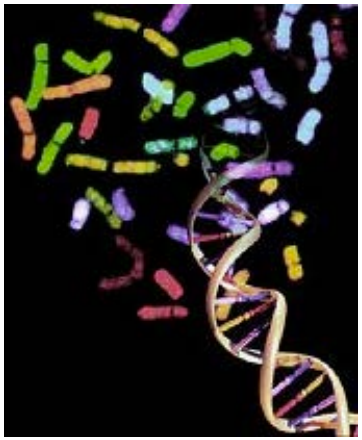
Évaluer le risque d'émergence de zoonoses à travers le prisme de l'immunoécologie et de l'immunogénétique



Les rongeurs et hantavirus responsables de fièvres hémorragiques

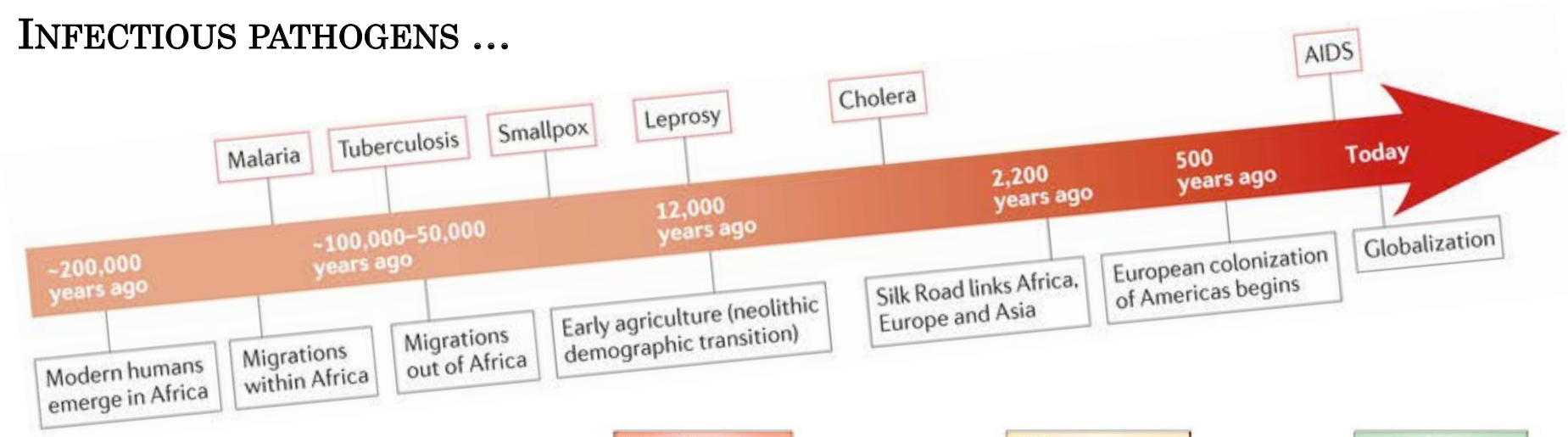
CBGP – 08 janvier 2019

nathalie.charbonnel@inra.fr

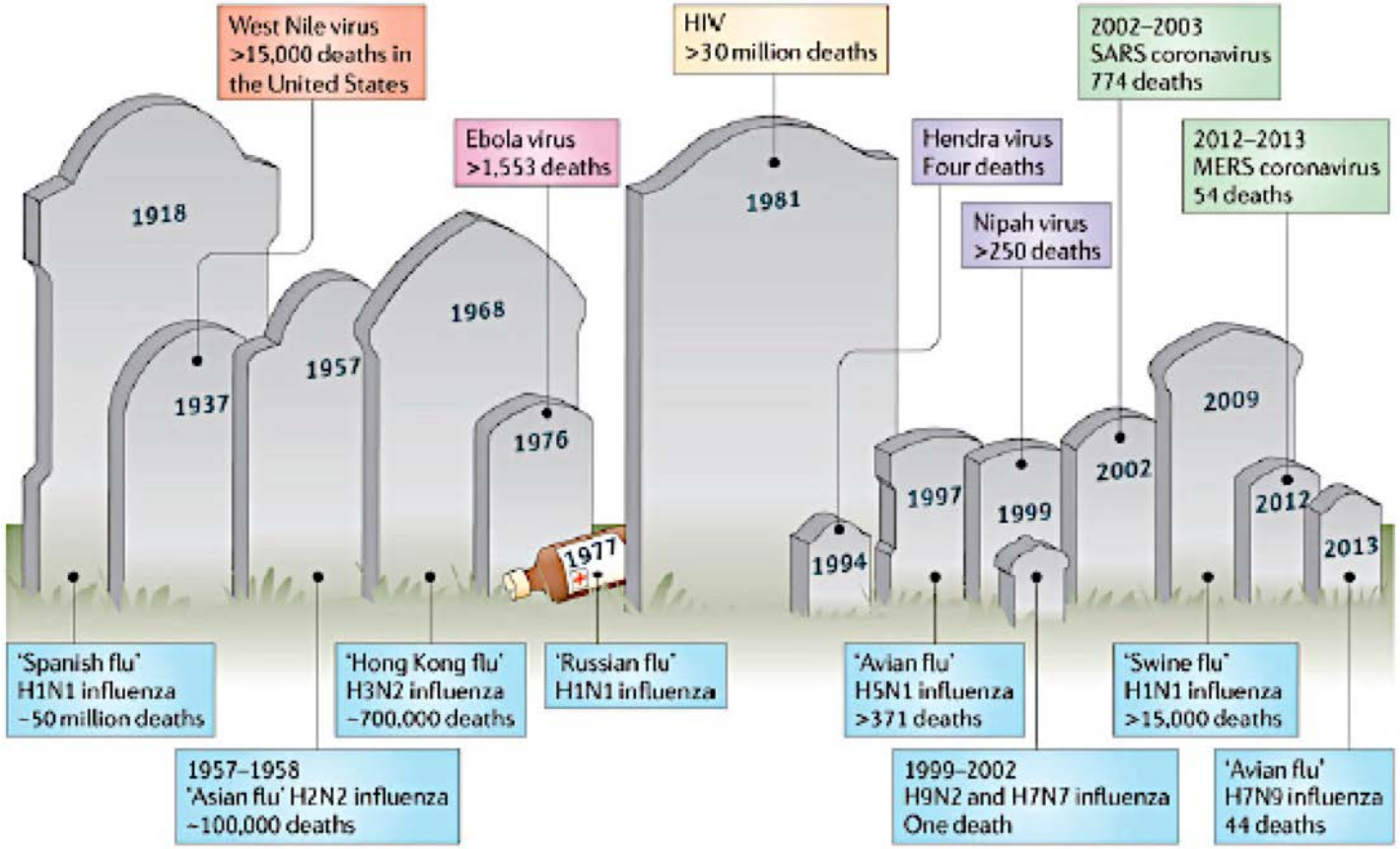

MERCI

CONTEXT

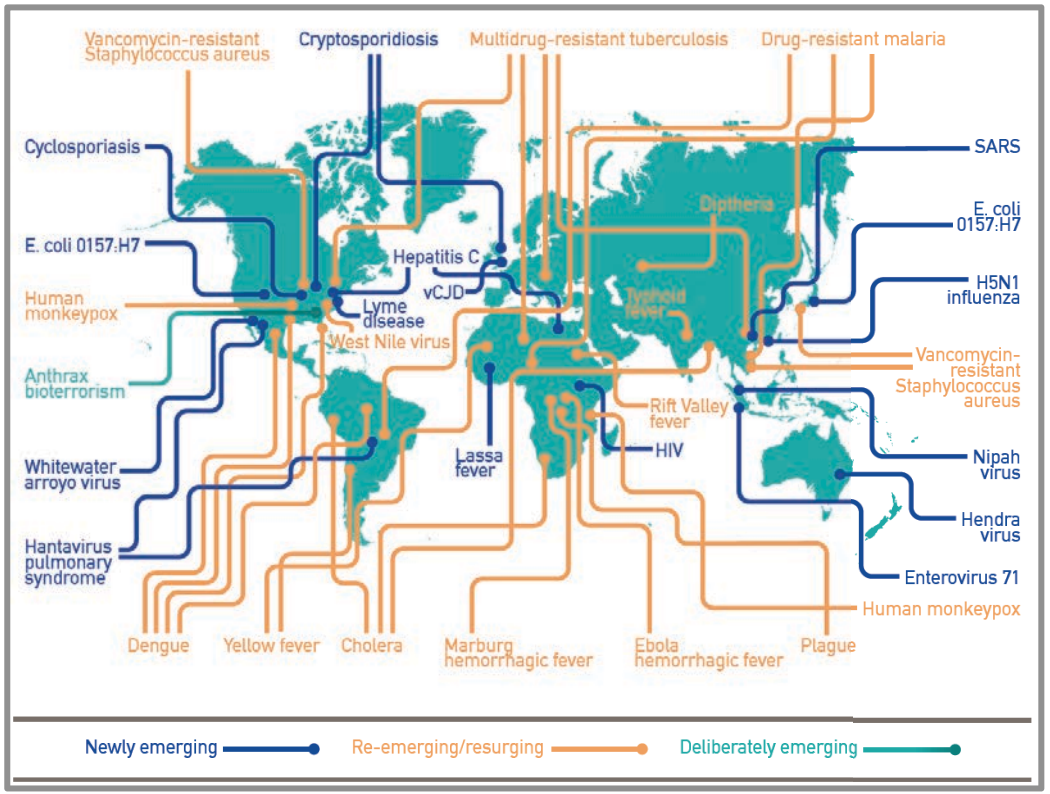
INFECTIOUS PATHOGENS …



Karlsson et al 2014

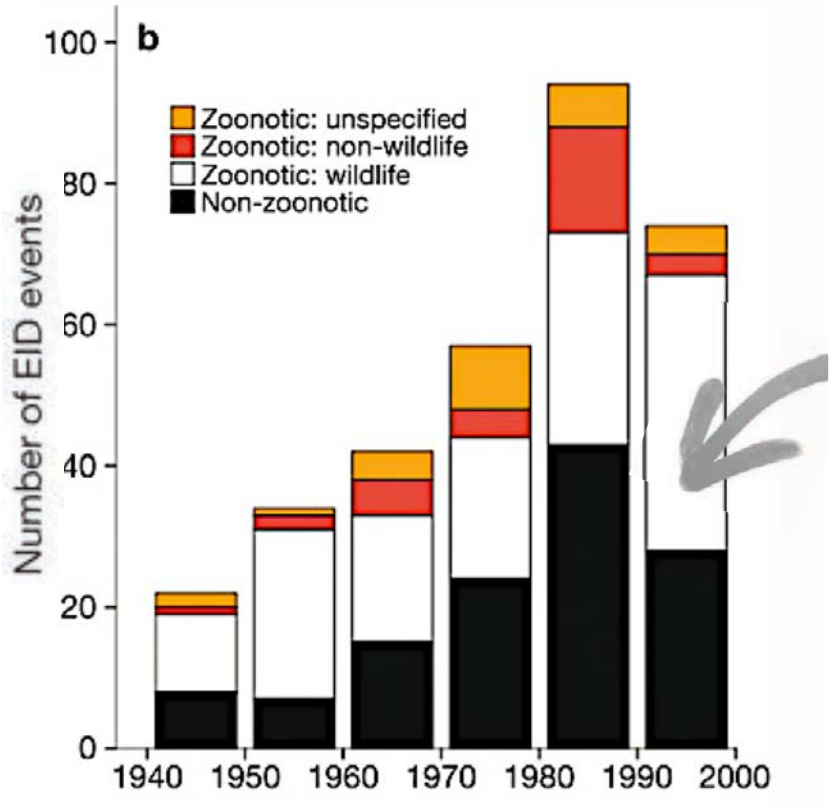


… EMERGING INFECTIOUS DISEASES



1 EID discovered every 8 months !

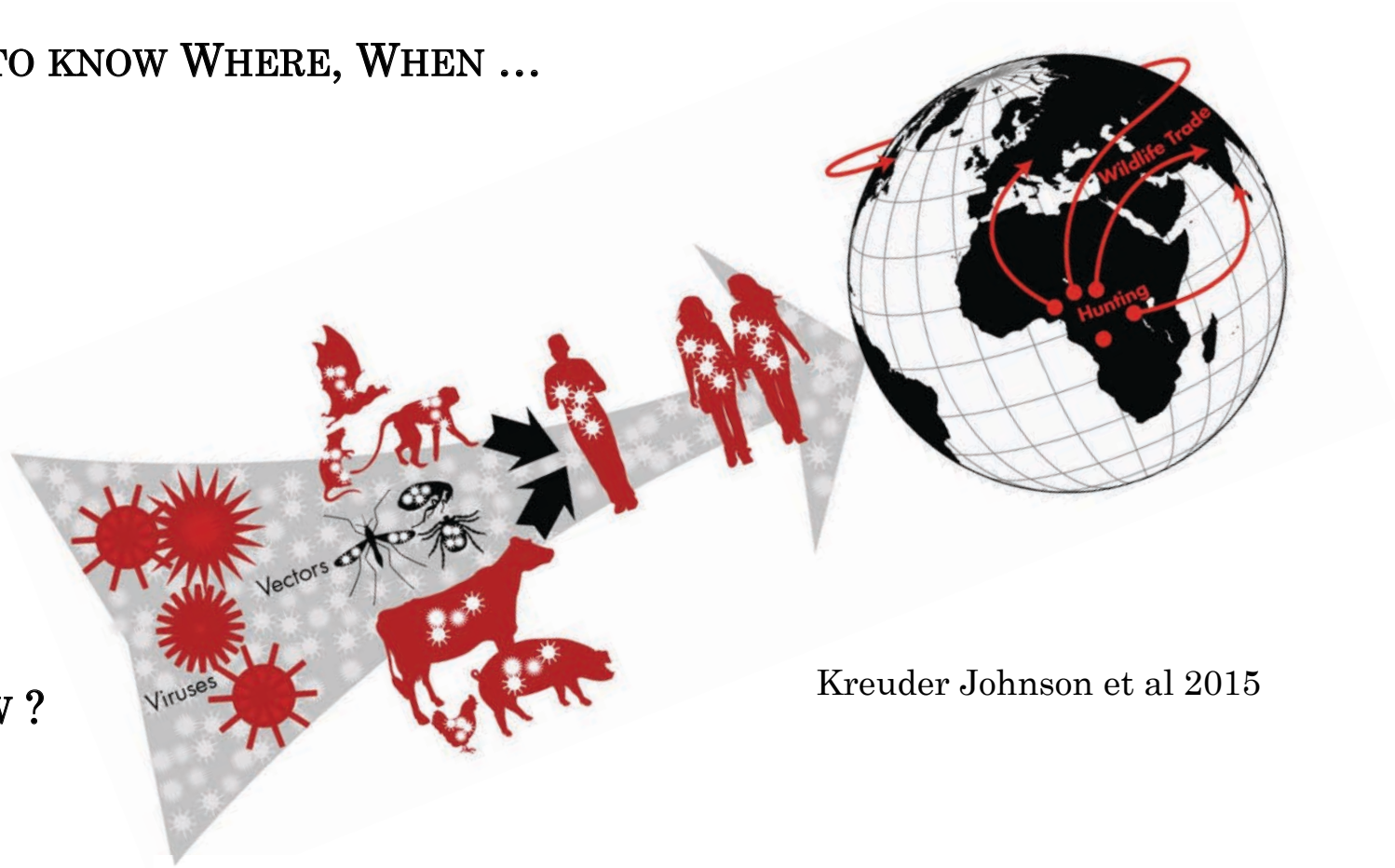
… and Zoonoses



Jones et al 2008

60% of EID are of animal origin
75 % are associated with wildlife

NEED TO KNOW WHERE, WHEN …



Kreuder Johnson et al 2015

… HOW ?

Reservoir Host Immune Responses to Emerging Zoonotic Viruses

Judith N. Mandl[1,7,*], Rafi Ahmed[2], Luis B. Barreiro[3], Pet…
Herbert W. Virgin[5], and Mark B. Feinberg[6]

Rodent reservoirs of future zoonotic diseases

Barbara A. Han[a,1], John Paul Schmidt[b], Sarah E. Bowden[b], and John M. Drake[b]

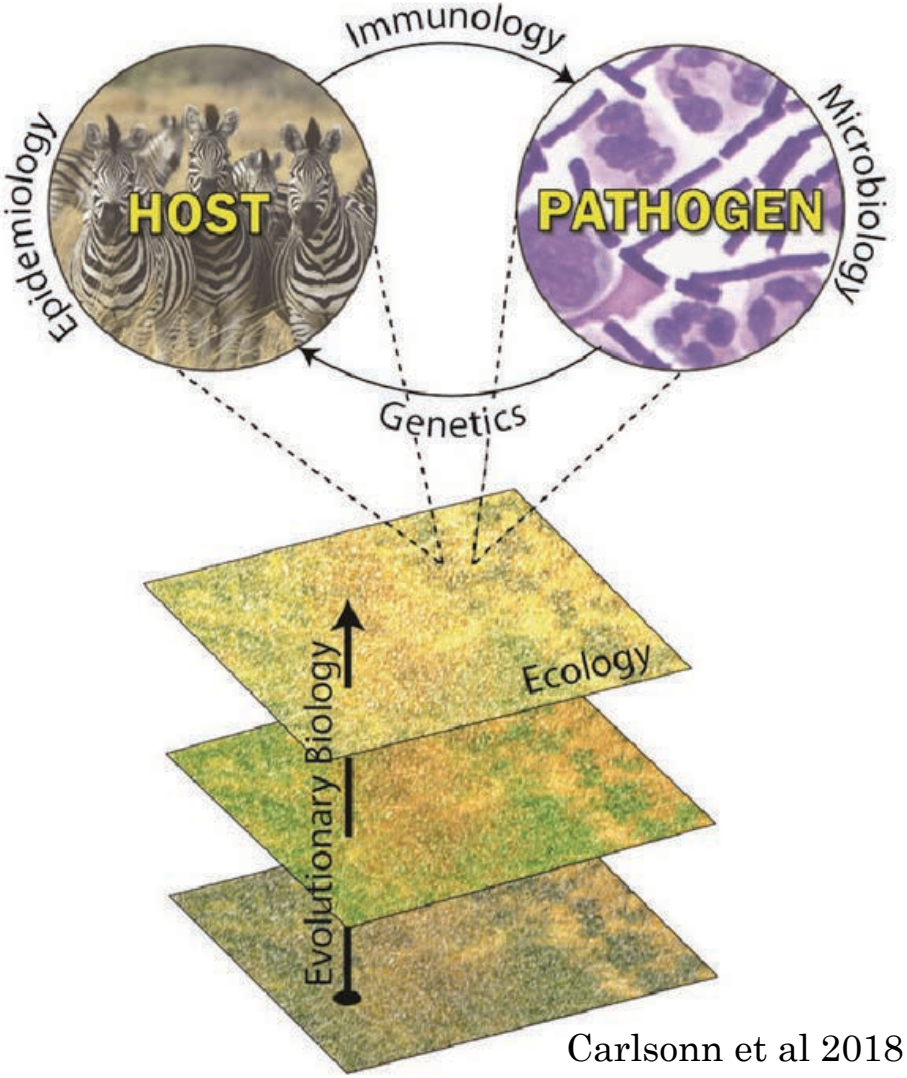
IMMUNE RESPONSE VARIABILITY

Need for studying immune variations

in an evolutionary ecology context

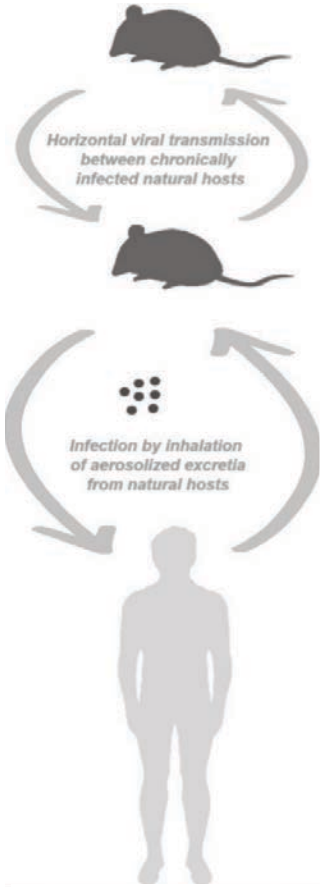
Between population variability

is still underlooked in wildlife



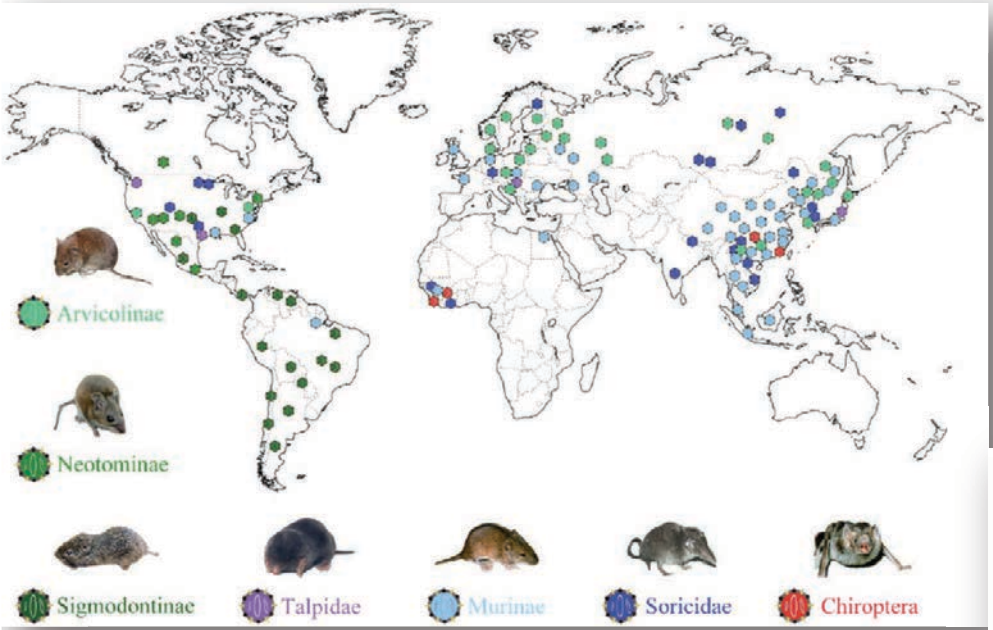
Carlsonn et al 2018

THE PARTICULAR CASE OF HANTAVIRUSES

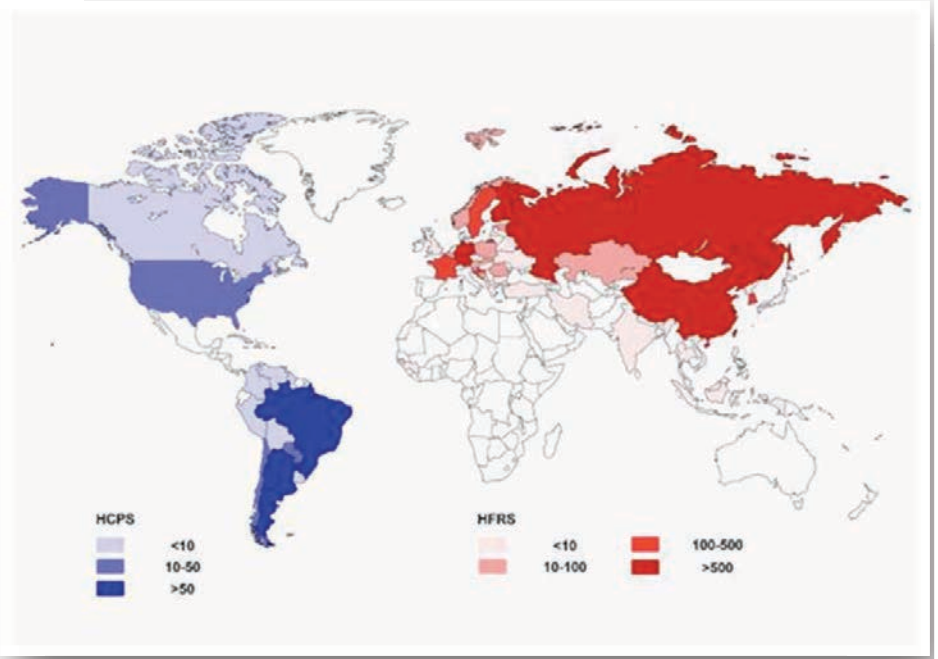


Hantavirus cycle

HETEROGENEOUS DISTRIBUTION OF HANTAVIRUS CASES WORLDWIDE

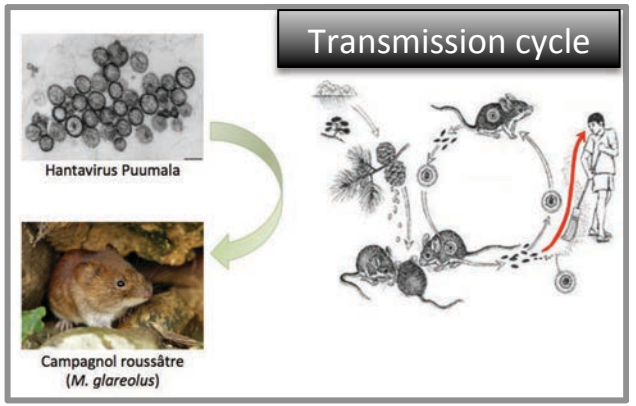


Guo et al. 2013



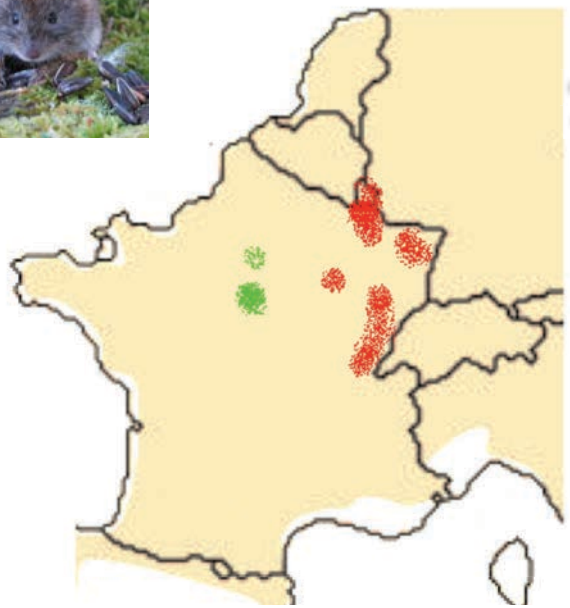
Jiang et al. 2017

HETEROGENEOUS DISTRIBUTION
Ex : NEPHROPATHIA EPIDEMICA CASES (PUUV HANTAVIRUS) IN FRANCE



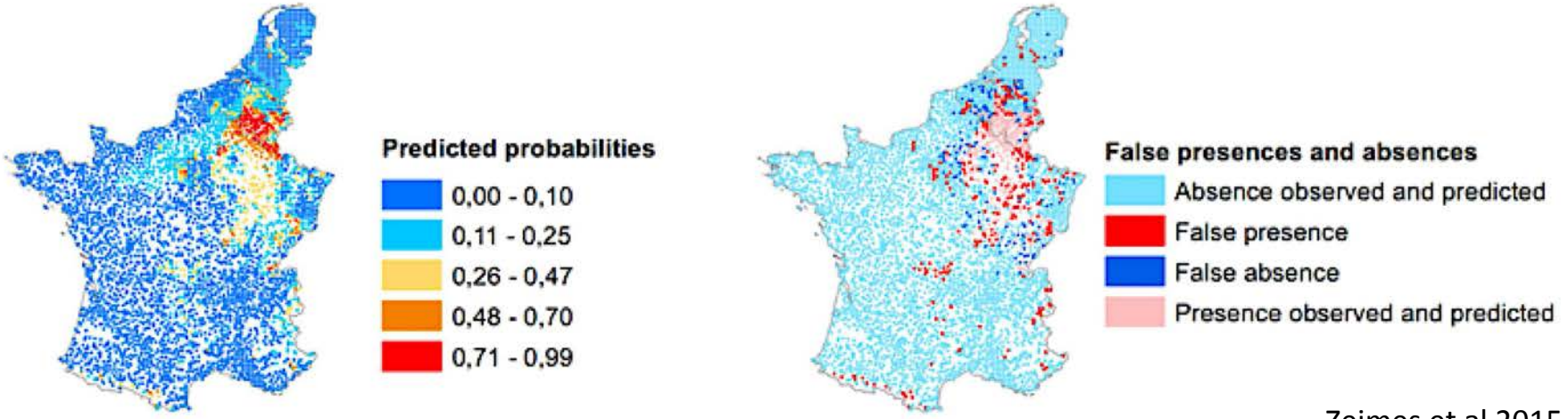
Human cases

PUUV in bank voles



Bank vole distribution

MODELING AND PREDICTING
WHERE AND WHEN SHOULD PUUV HUMAN CASES OCCUR



Zeimes et al 2015

Abiotic factors (climate, environmental features) do not alone enable to predict the distribution of PUUV

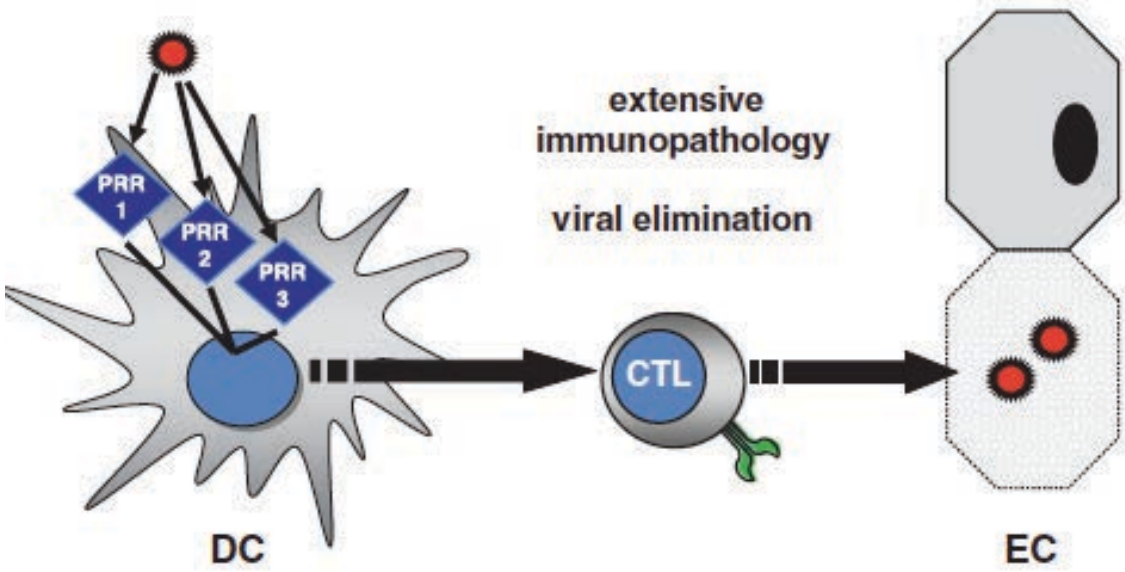
Do biotic factors (host, virus, pathobiome) improve our understanding of the distribution and risk of PUUV ?

THE IMMUNE PERSPECTIVE …

In humans

No replication of hantaviruses

Pulmonary / renal syndroms due to immunopathologies



Higher than normal numbers of interleukin (IL), tumor necrosis factor (TNF), interferons (INF), and activated Tc.

Hantavirus pathogenesis is a result of the host inflammatory response to the virus.

Schönrich et al 2018

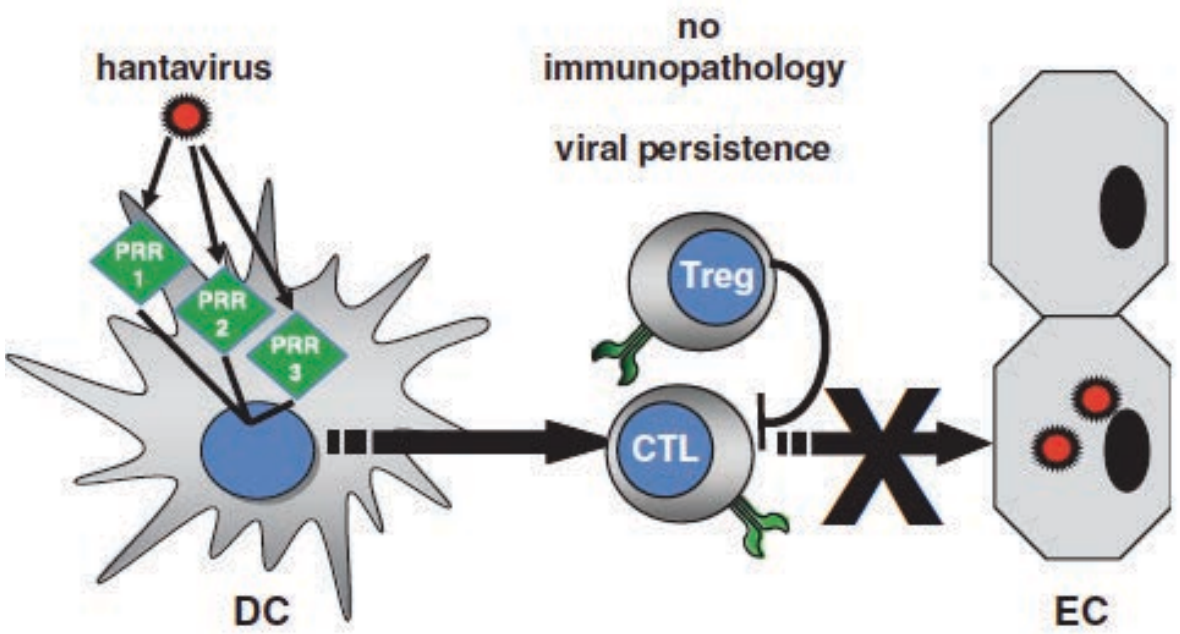
In reservoirs

Long co-adaptation

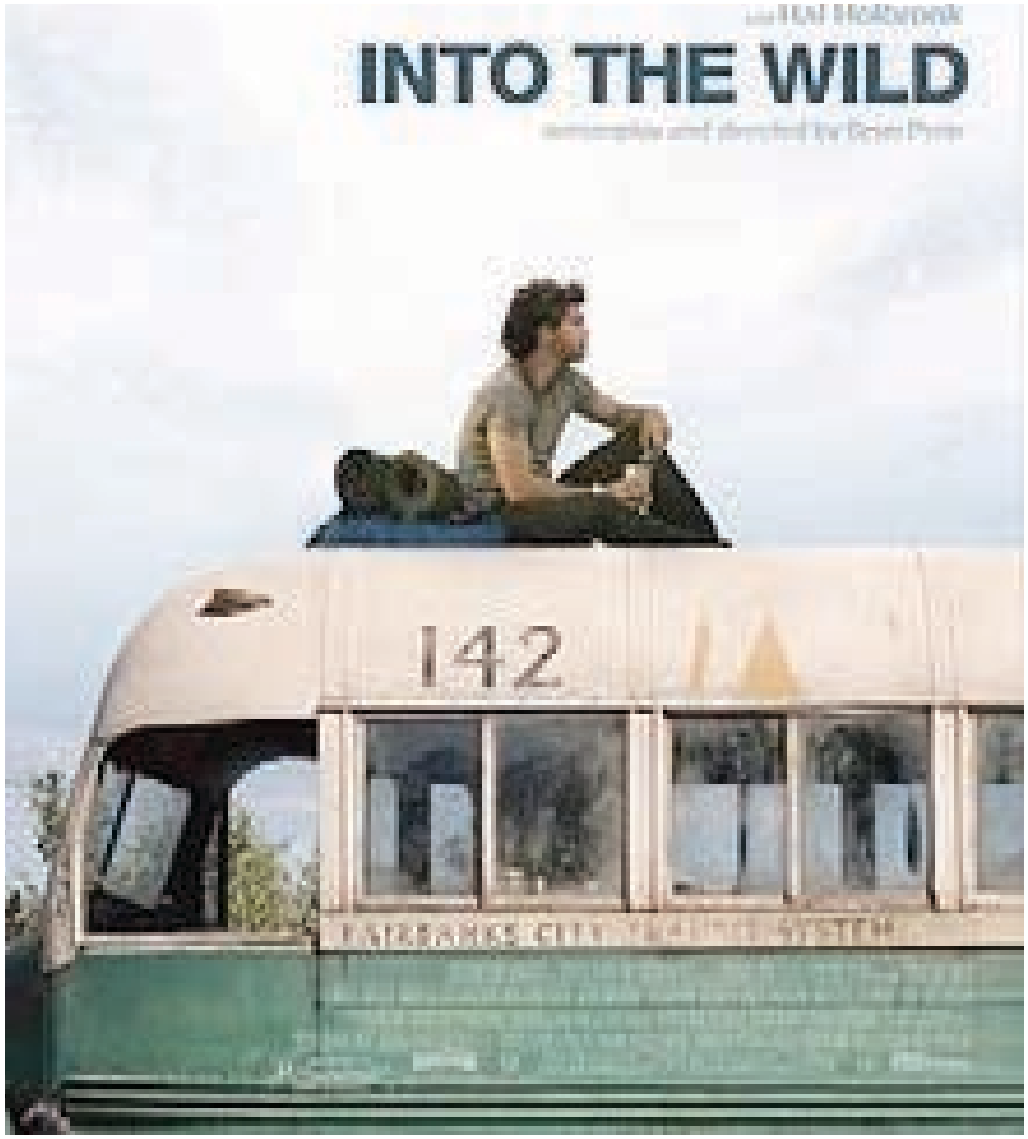
⇳

Tolerance to hantavirus ?

Chronic
and ≅ asymptomatic infection



THE IMMUNE PERSPECTIVES …



THE IMMUNE PERSPECTIVES …

Spatial variability in bank vole responses (tolerance) to PUUV ?

Immunology

Signature of this variability in bank vole genomes ?

Immunogenetics and genomics

Influence of virus genetic variability or pathobiome on bank vole responses ?

Epidemiology

Links with PUUV distribution ?

HYPOTHESES

Evolution of Resistance
Costs of infection > Immunopathologies

Evolution of Tolerance
Costs of infection < Immunopathologies

Lower prevalence
Lower PUUV load

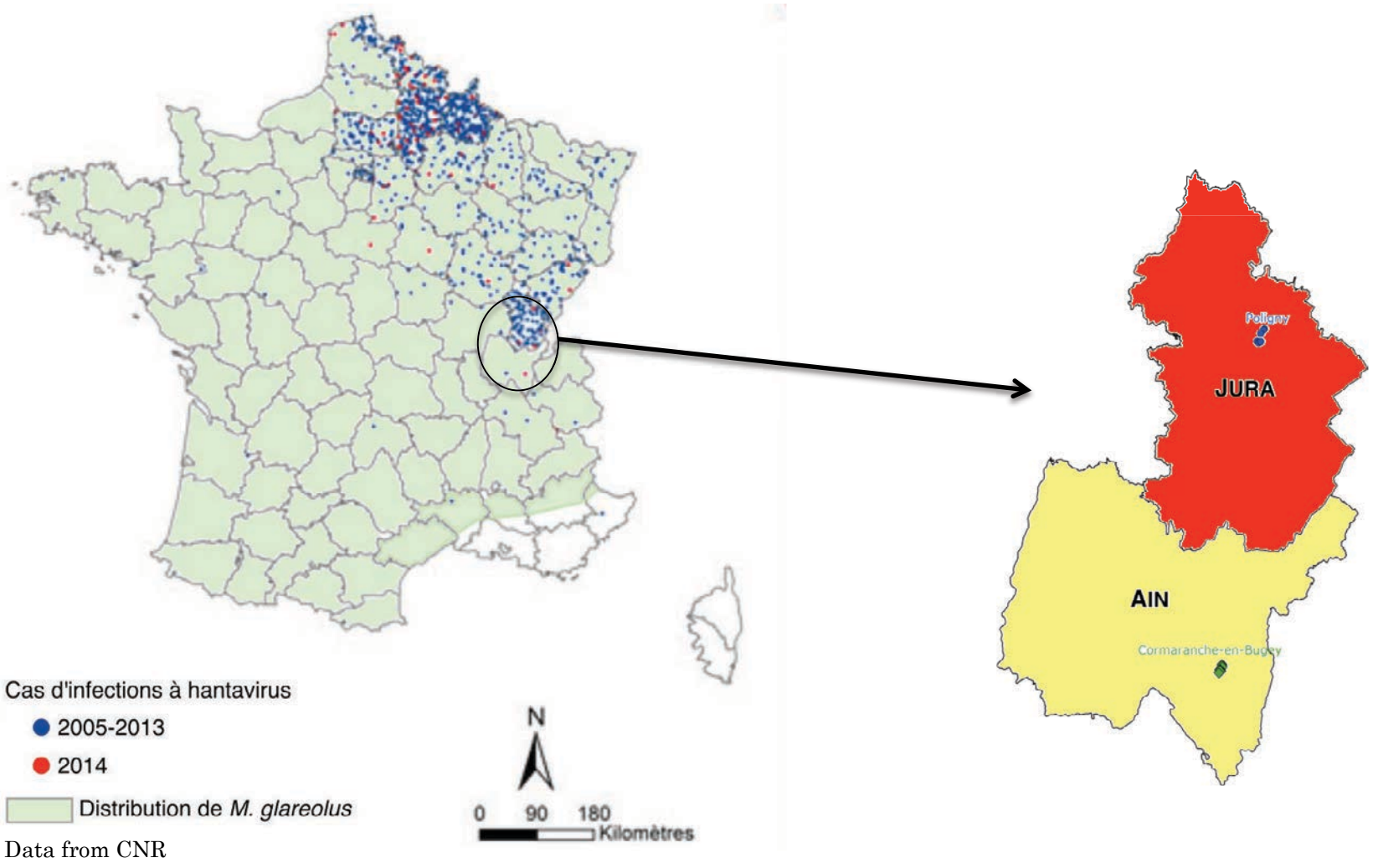
Lower risk of human transmission

Higher prevalence
Higher PUUV load

Higher risk of human transmission

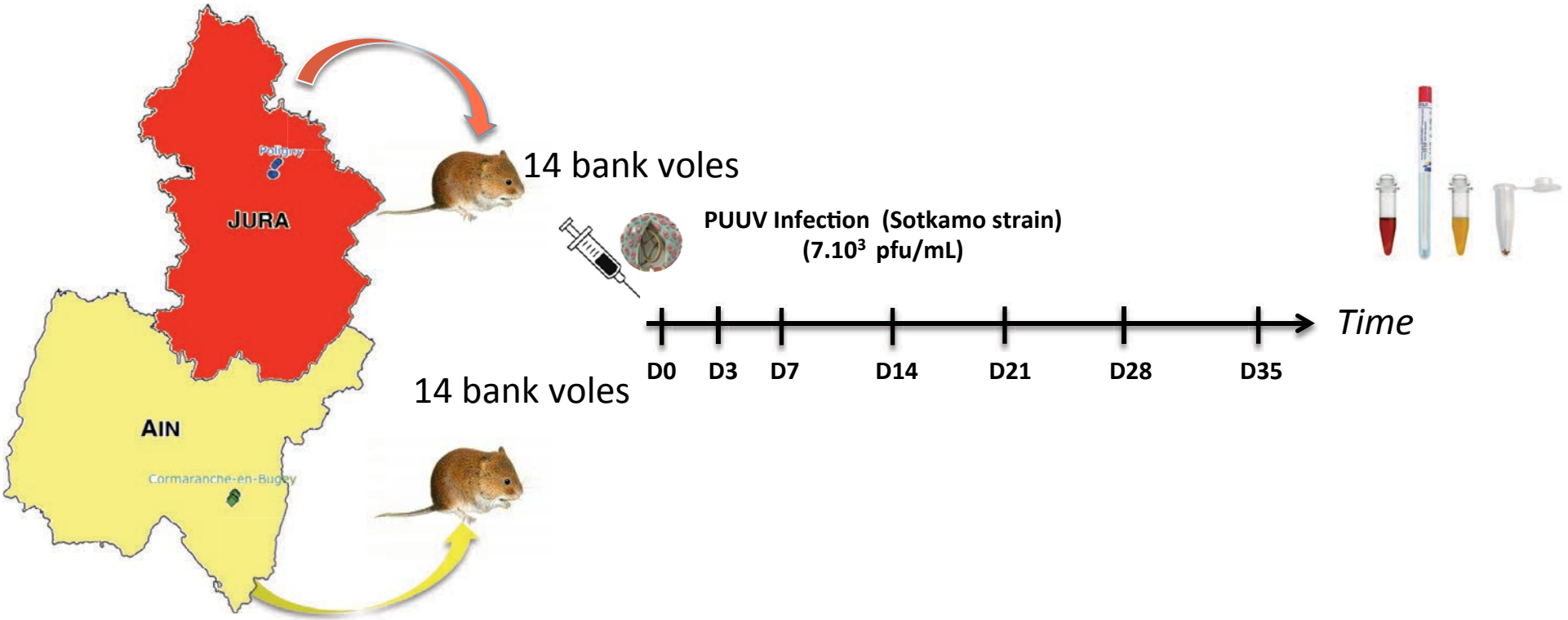
IMMUNOLOGICAL APPROACHES

COMPARISON OF BANK VOLE IMMUNE RESPONSES BETWEEN PUUV ENDEMIC AND NON ENDEMIC AREAS



Data from CNR

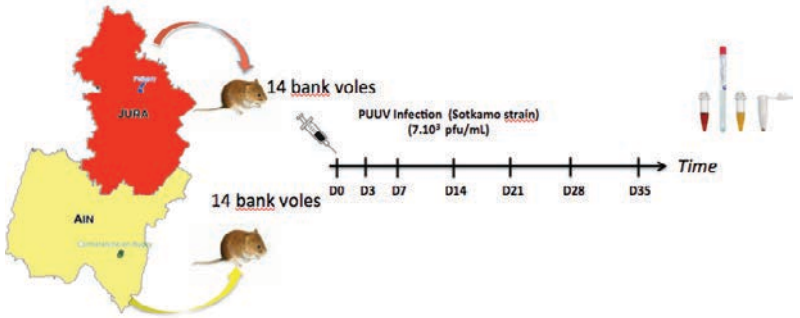
EXPERIMENTAL INFECTIONS



Collab. ANSES Lyon

EXPERIMENTAL INFECTIONS

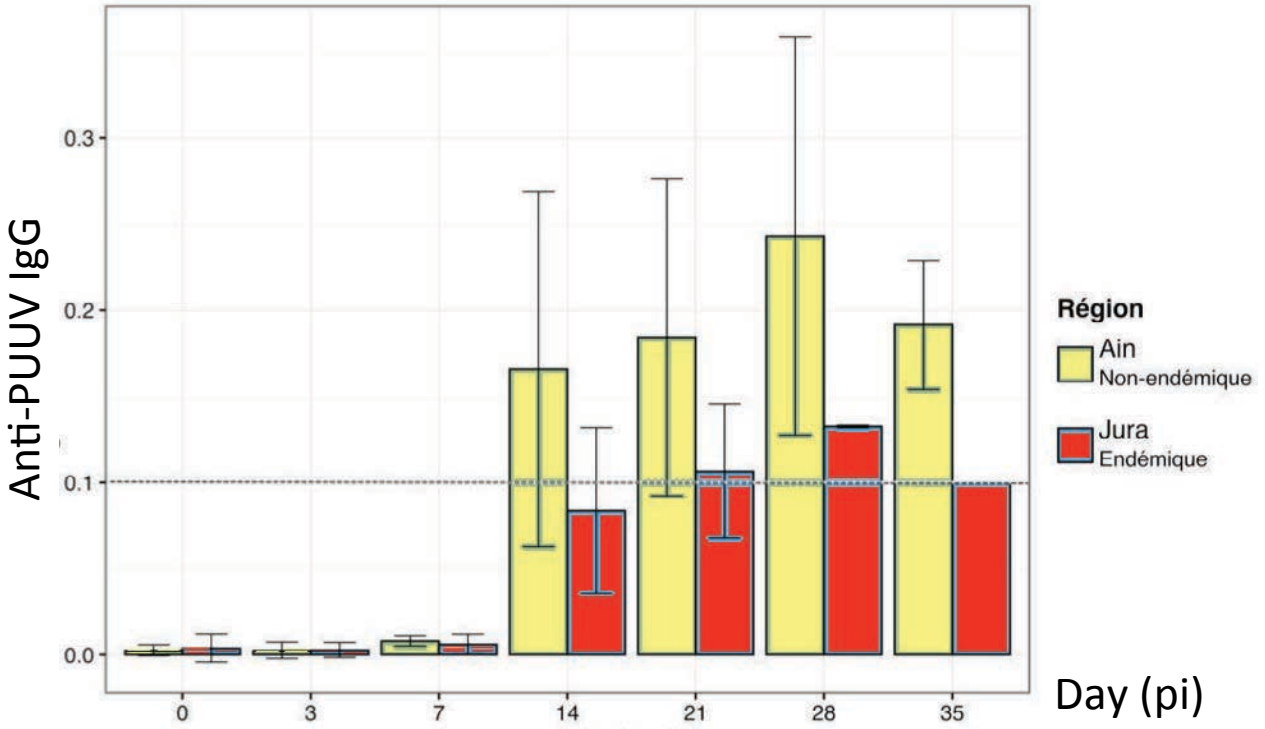
1- ANTIBODY PRODUCTION



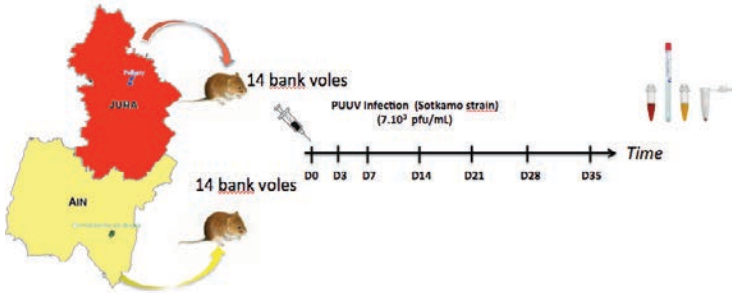
Inter-individual variability

All voles seroconverted -> Bank voles from the non endemic area are sensitive to PUUV

Higher & earlier production of antibodies in the non endemic region



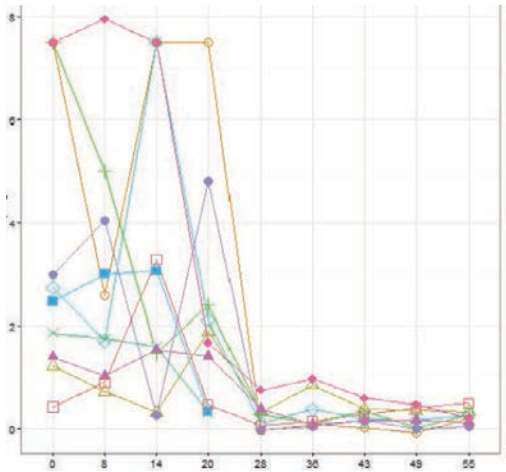
2- Inflammatory response



High inter-individual variability of the inflammatory response

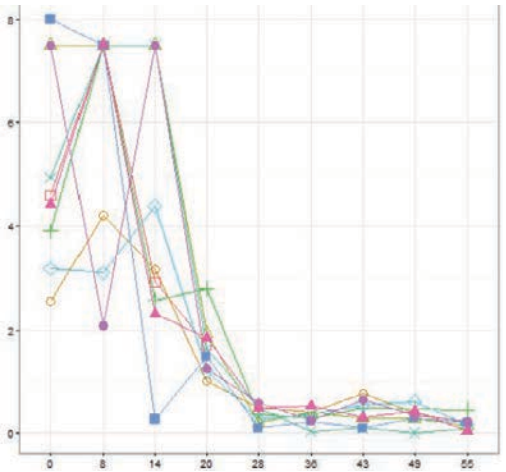
Longer inflammatory response of voles from Ain

Ain



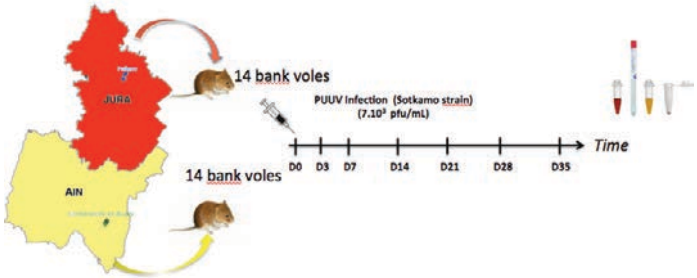
Day (pi)

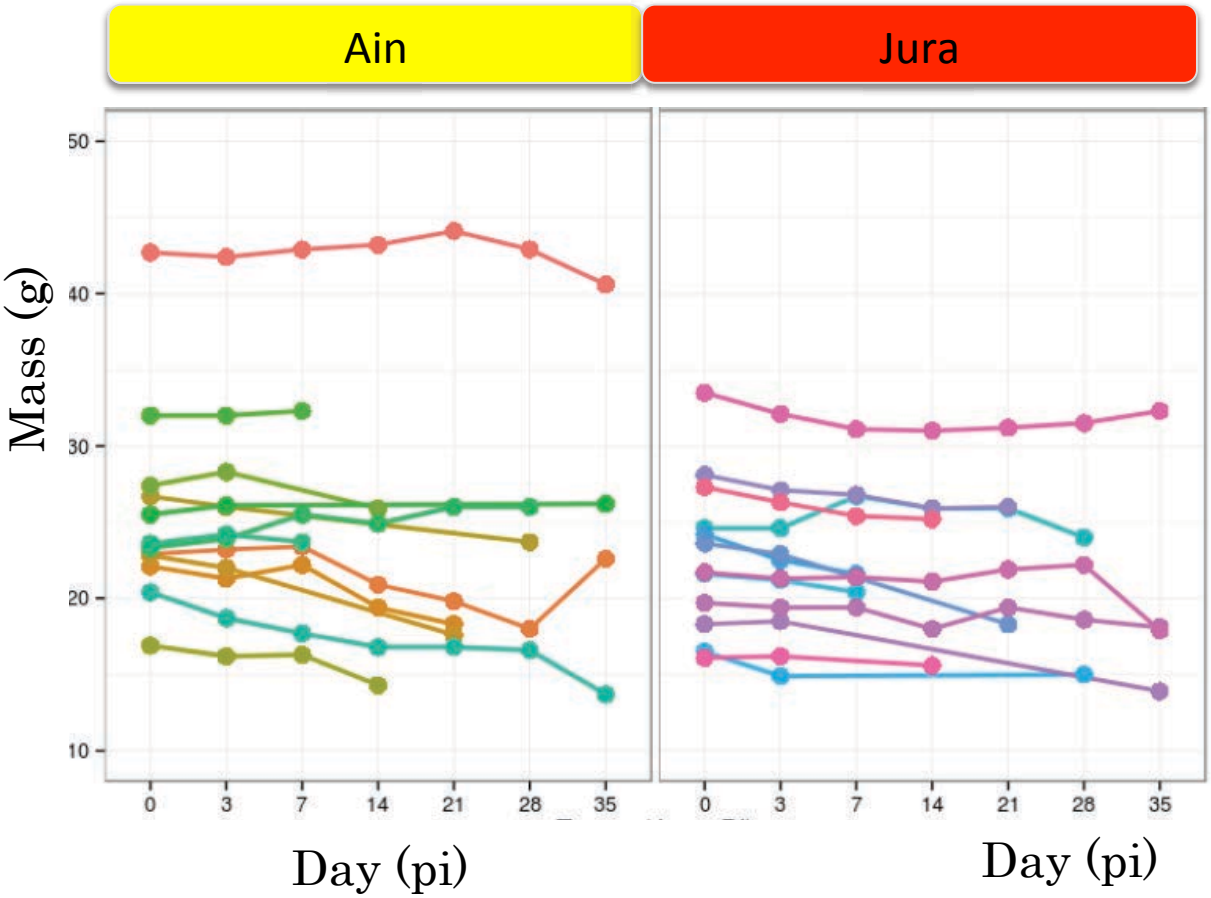
Jura



Day (pi)

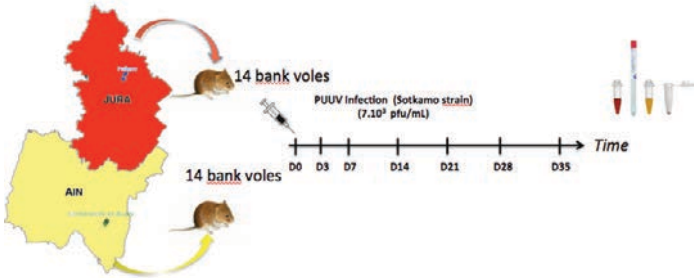
3- Difficulty to assess tolerance …

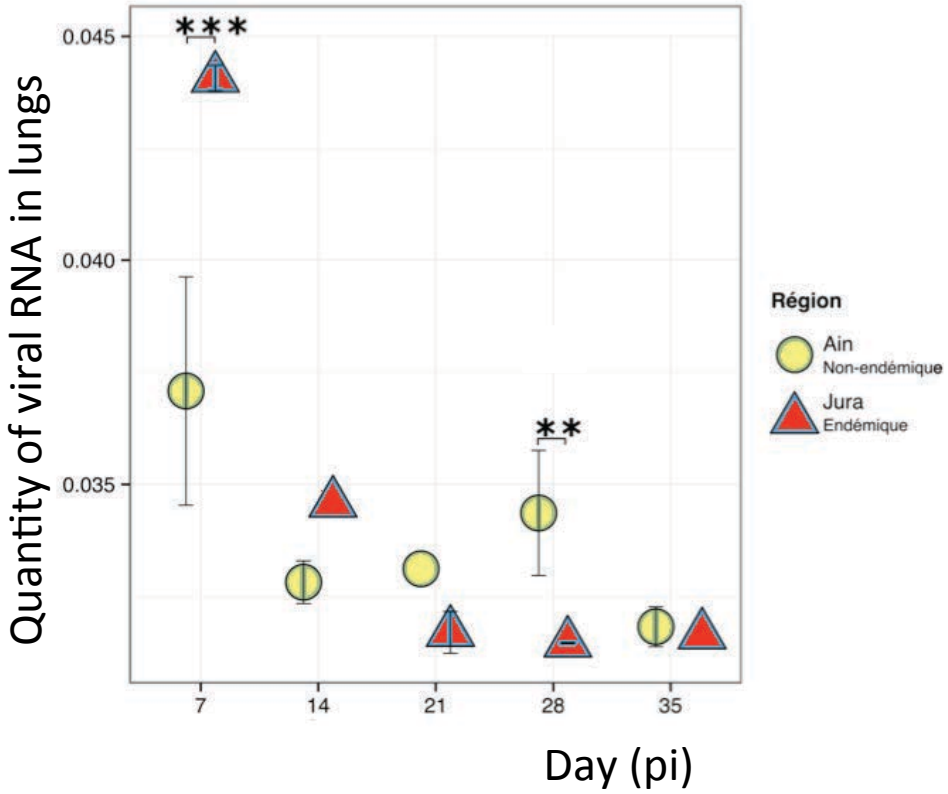




No changes of temperature or body mass
No evidence of tolerance with these parameters

4- Impact of bank vole immune response on PUUV replication





High inter-individual variability of PUUV viral load detected in target organs

Higher replication of PUUV for infected bank voles of the endemic region

Differences in Resistance / Tolerance ?

PUUV INFECTION WITH A WILD PUUV FRENCH STRAIN (ARDENNES)

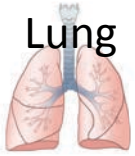
Ardennes

Endemic region

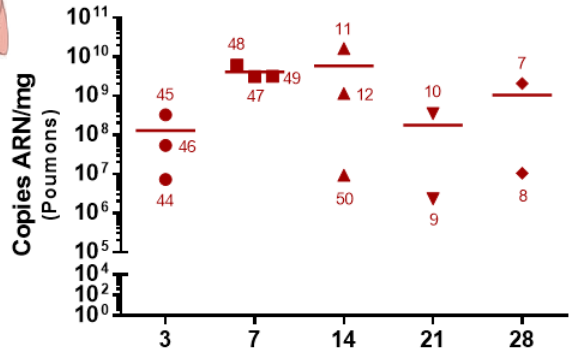
Loiret

Peri endemic region

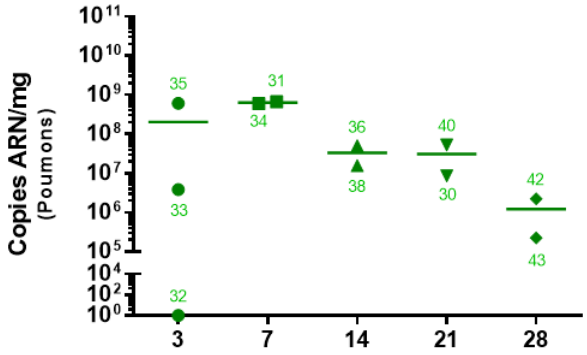
Lung



Quantity of viral RNA



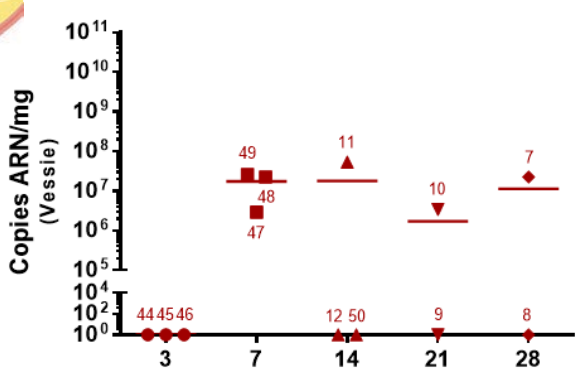
Day (pi)



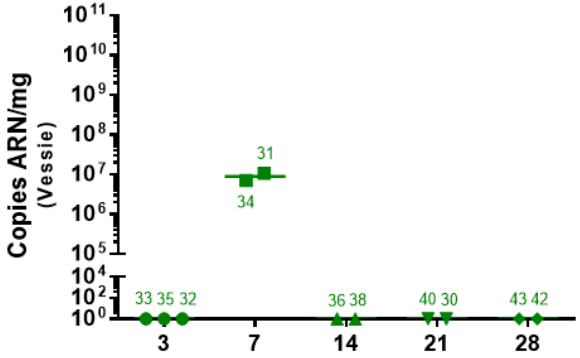
Day (pi)

Bladder

Quantity of viral RNA



Day (pi)



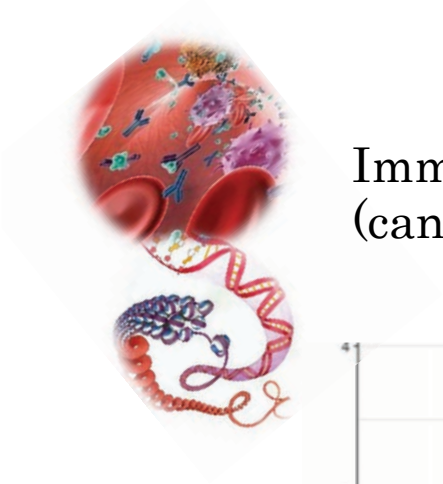
Day (pi)

Higher and longer rate of PUUV replication for bank voles of the endemic region

Lower Resistance ?
Super-spreaders ?

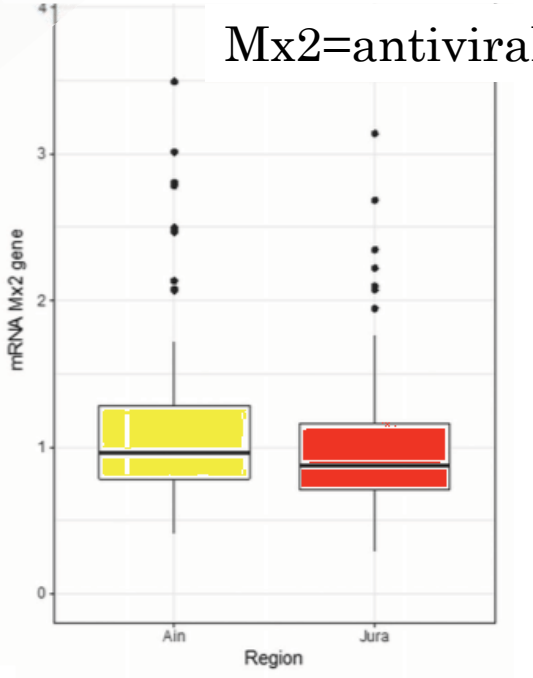
Local adaptation of PUUV ?
Cross-infection in progress

VARIABILITY OF IMMUNE RESPONSES IN NATURAL POPULATIONS

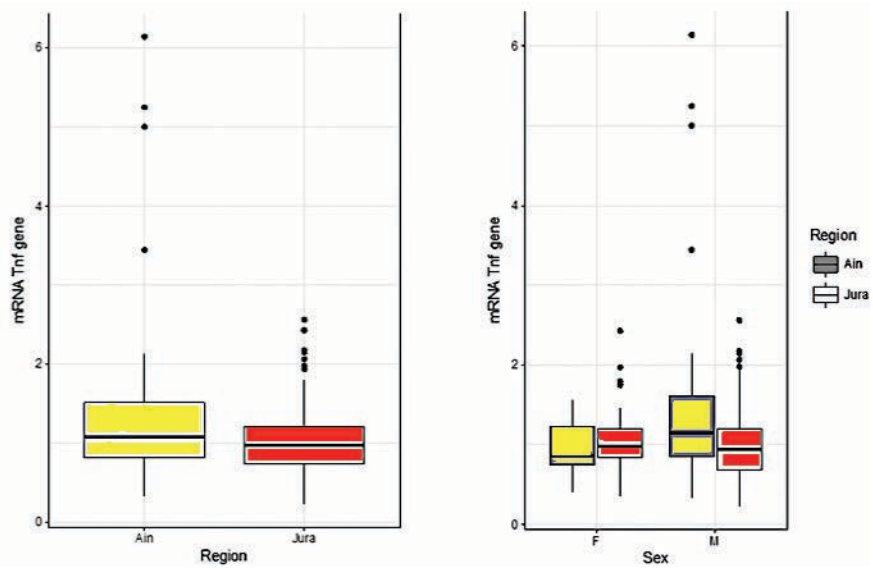


Immune phenotype = Immune gene expression
(candidate genes chosen from the literature)

Mx2=antiviral

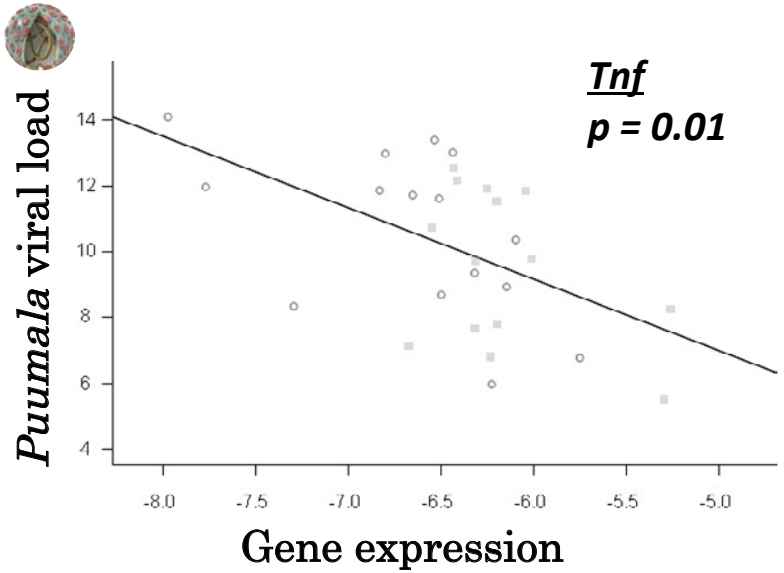


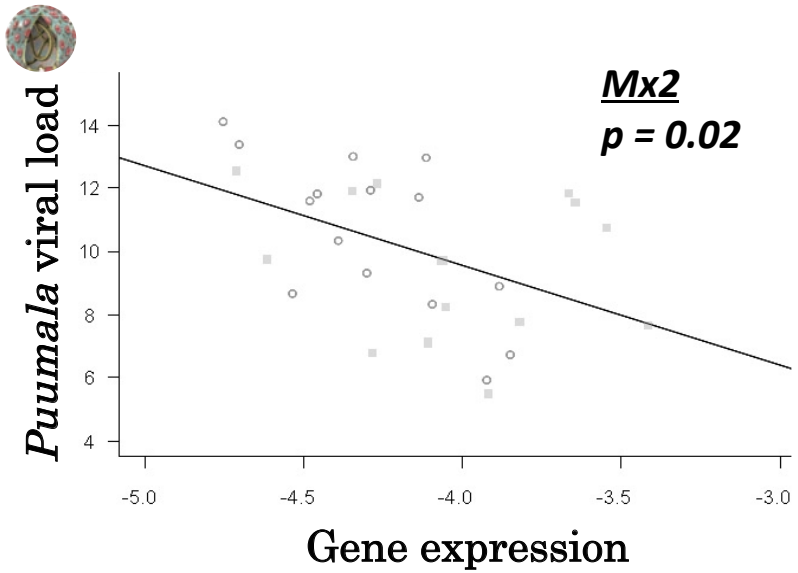
Tnf=pro-inflammatory



Higher immune gene expression in the non endemic compared to the endemic region

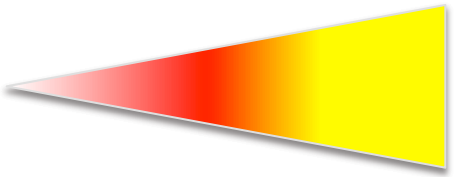
French Ardennes Study





Strong negative relationships
between immune gene expression (inflammation, antiviral)
and PUUV replication

Tolerance



Resistance

CONCLUSIONS FROM THE IMMUNOLOGICAL APPROACHES

Bank voles from non endemic areas are sensitive to infections with PUUV lab or wild strains

There is a high inter-individual variability in the response to PUUV

There is a slight inter-populational variability in the response to PUUV

- Higher antibodies and longer inflammation in bank voles from non endemic area
- Weaker replication of PUUV in bank voles from non endemic area

Higher **resistance** of bank voles from non endemic areas ?
Higher **tolerance** of bank voles from endemic areas ? Super-spreaders ?

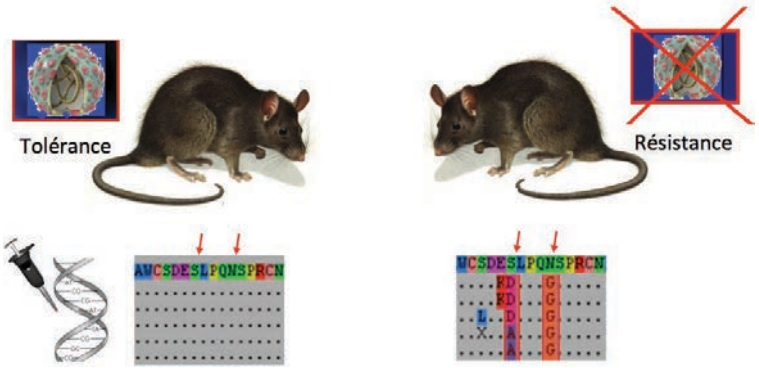
There is a risk for Hantavirus disease emergence
in non endemic areas adjacent to endemic ones.

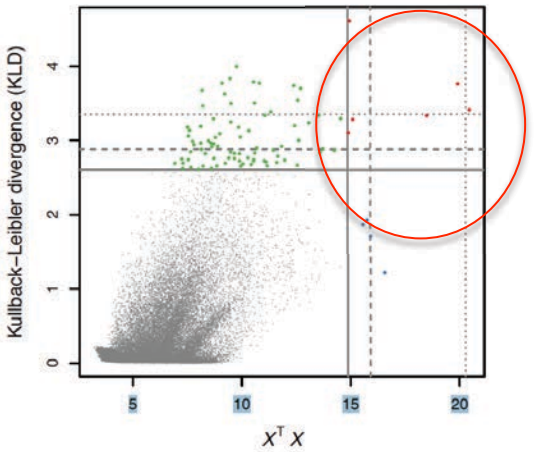
The risk might be limited by a strong immune response against PUUV,
leading to its rapid elimination in bank voles,
what should limit its persistence in these reservoir populations

Can tolerance to PUUV evolve in these non endemic areas adjacent to endemic ones ?

IMMUNOGENETICS & GENOMICS APPROACHES

PRINCIPLES OF ADAPTATION GENOMICS

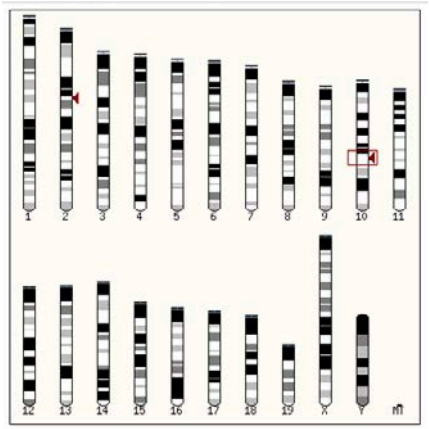




Identification of 'outliers' loci

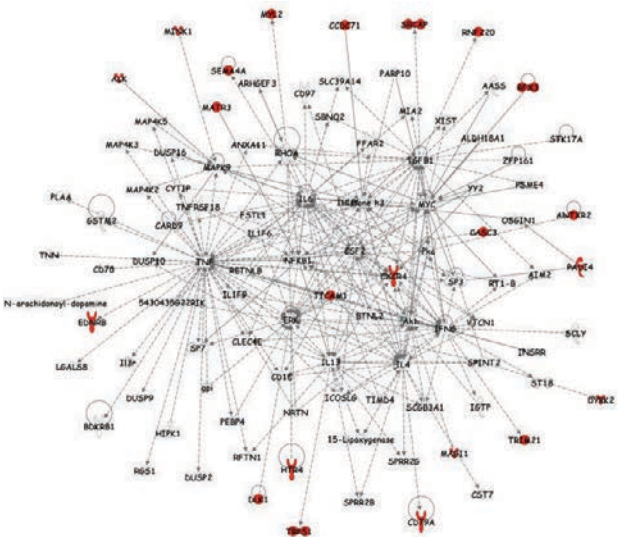
Higher genetic differentiation than expected under neutral models

Genome scan :
Identification of genes evolving under positive directional selection between populations

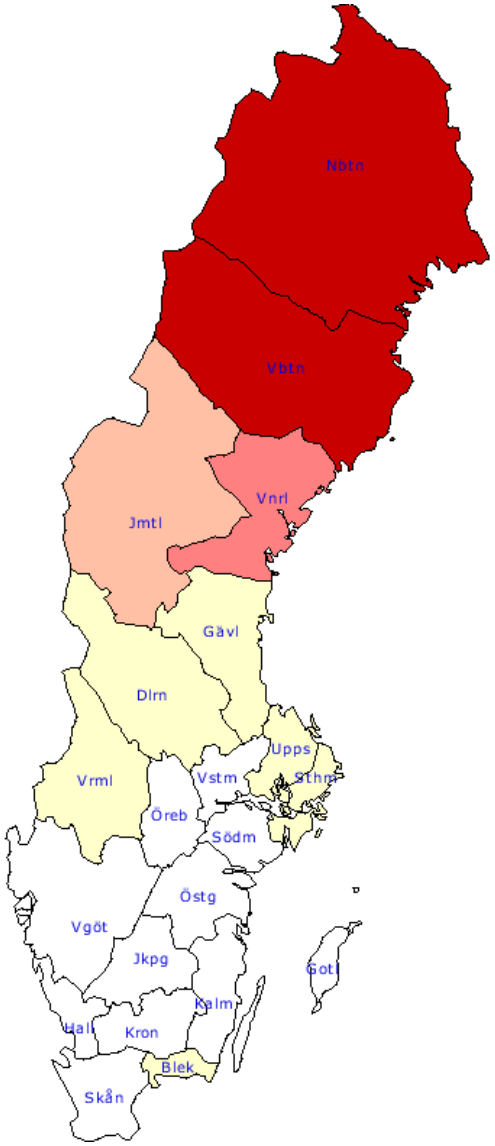


Annotation of outliers
Gene ontology

Enrichment analyses
Network analyses



SPATIAL DISTRIBUTION OF NEPHROPATHIA EPIDEMICA IN SWEDEN



Sweden

- High presence of human cases
- Low presence of human cases
- Absence of human cases

Human socio-economics

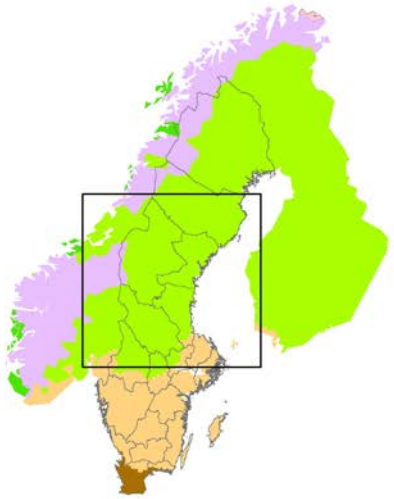
Probability of human exposure to NE

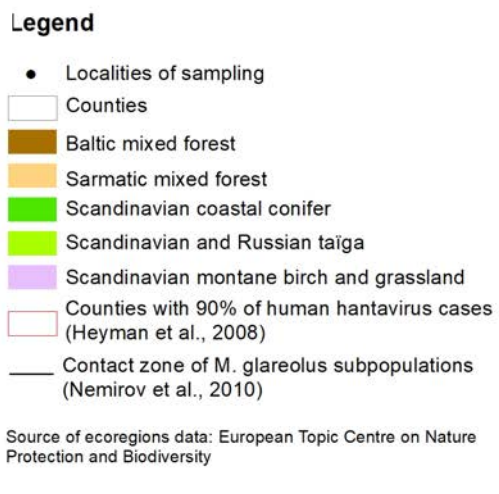
Environmental variables

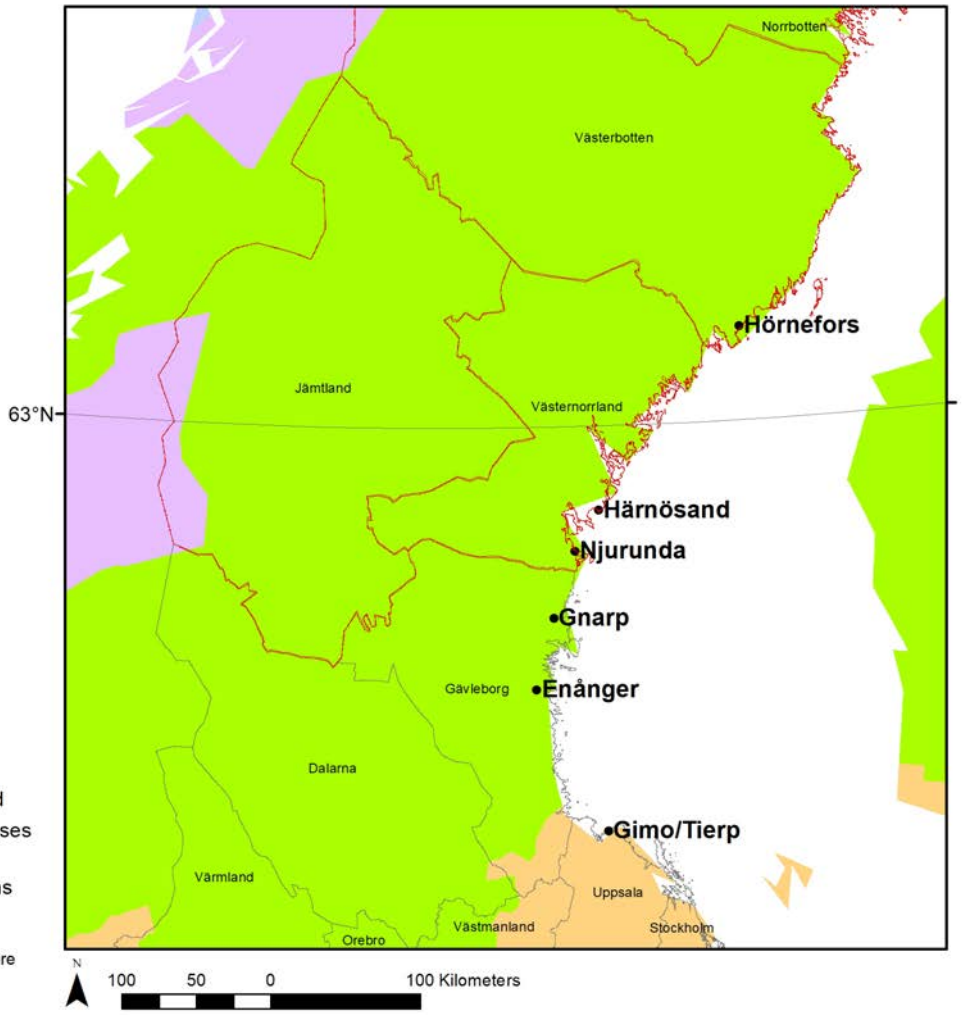
PUUV persistence in the environment
Bank vole population dynamics

Bank vole population heterogeneity
Immunity, genomics

BANK VOLE POPULATION SAMPLING







Collab. SLU Sweden

Gene candidate approach

based on the medical literature

Tnf, Mx2, TLR4, TLR7 (SNP genotyping, Kaspar technology)

but restrictive approach (you find only what you search)

Whole genome approach

Blind, no a priori - but *Myodes glareolus* has a large genome (3Gb)

Need for a reduced representation sequencing : RAD-sequencing

Enzyme : SbfI = 8 cutter

Pools of 35/40 individuals

6 pops, 4 replicates (Mid tags 5 & 6 bases, 2 librairies)

Paired-end sequencing, 2x100 bp

70,000 contigs, 500 bp

95,000 SNPs (> 40 reads, <5% singleton)

DETECTION OF SIGNATURE OF SELECTION

1- Population Genomic Differentiation

SelEstim

Detecting and Measuring Selection from Gene Frequency Data

Renaud Vitalis,*,†,1 Mathieu Gautier,*,† Kevin J. Dawson,‡ and Mark A. Beaumont§
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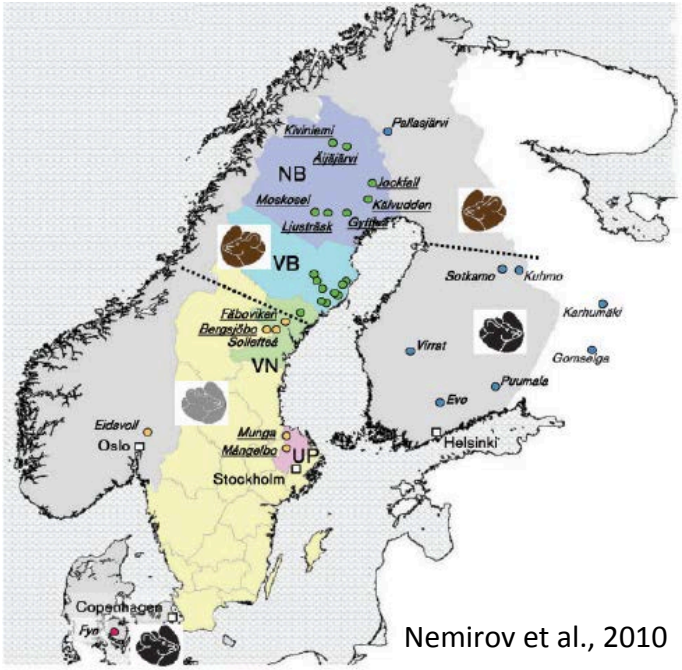

Nemirov et al., 2010

High Fst estimates,
Hierarchical structure

BayPass

Genome-Wide Scan for Adaptive Divergence and Association with Population-Specific Covariates

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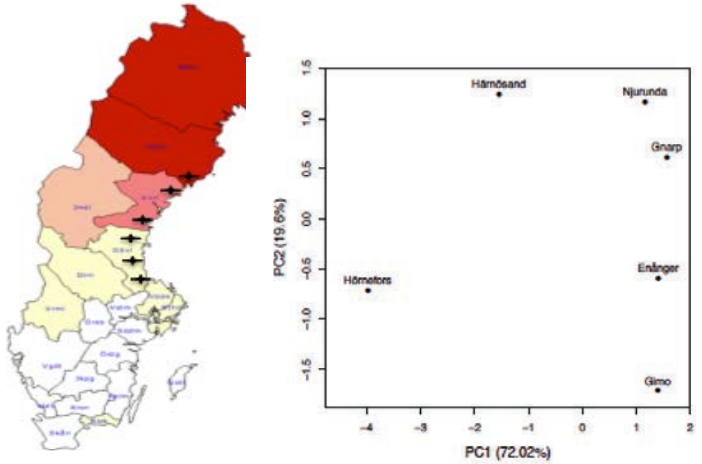

2- Environmental & climatic variables

Genome-Wide Scan for Adaptive Divergence and Association with Population-Specific Covariates

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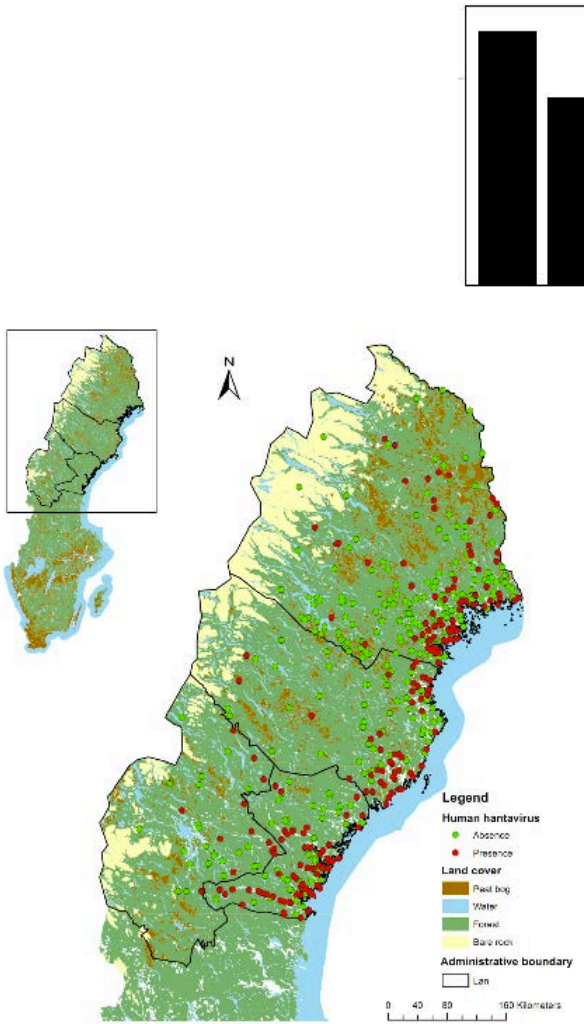
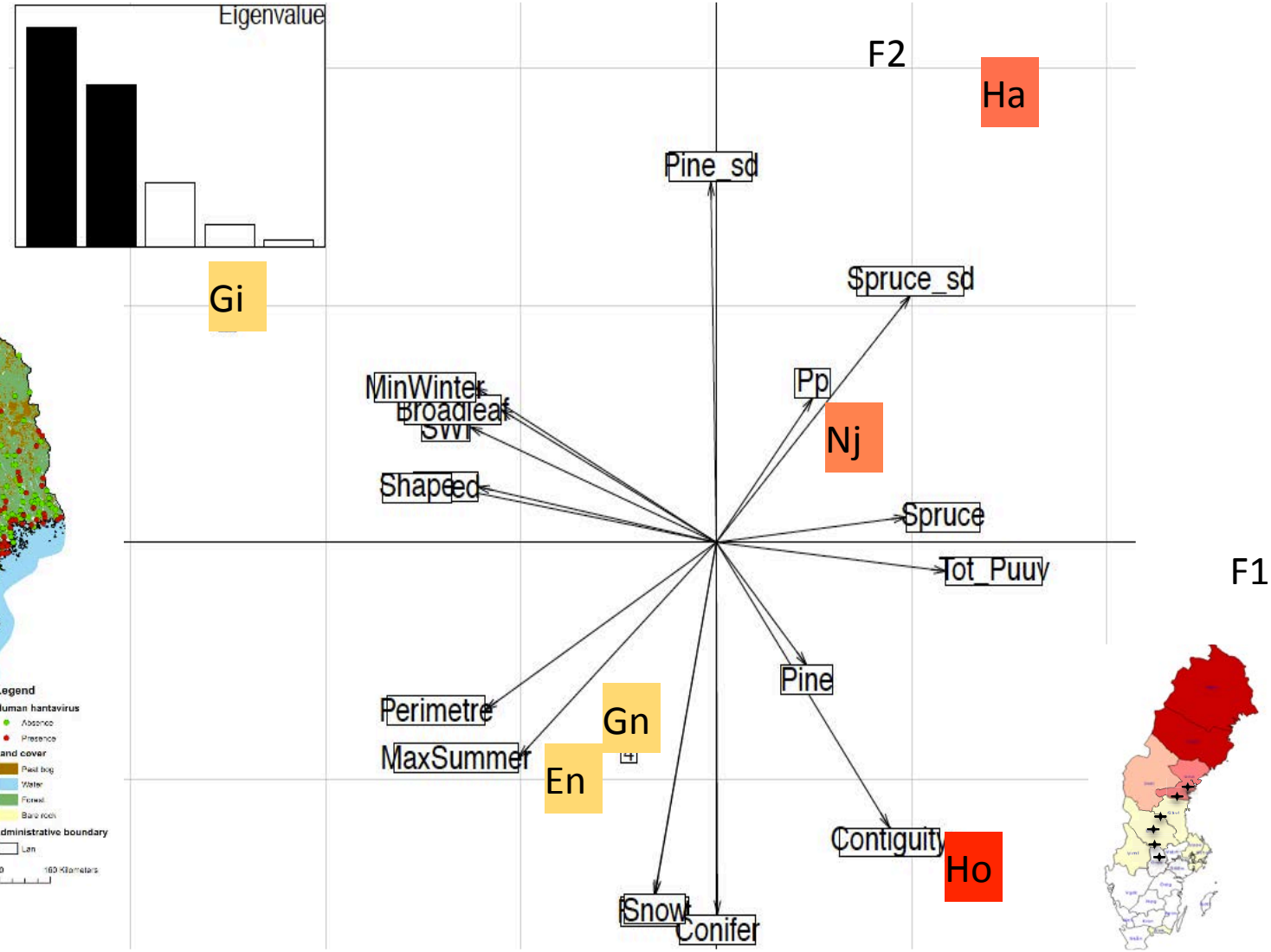
BayPass

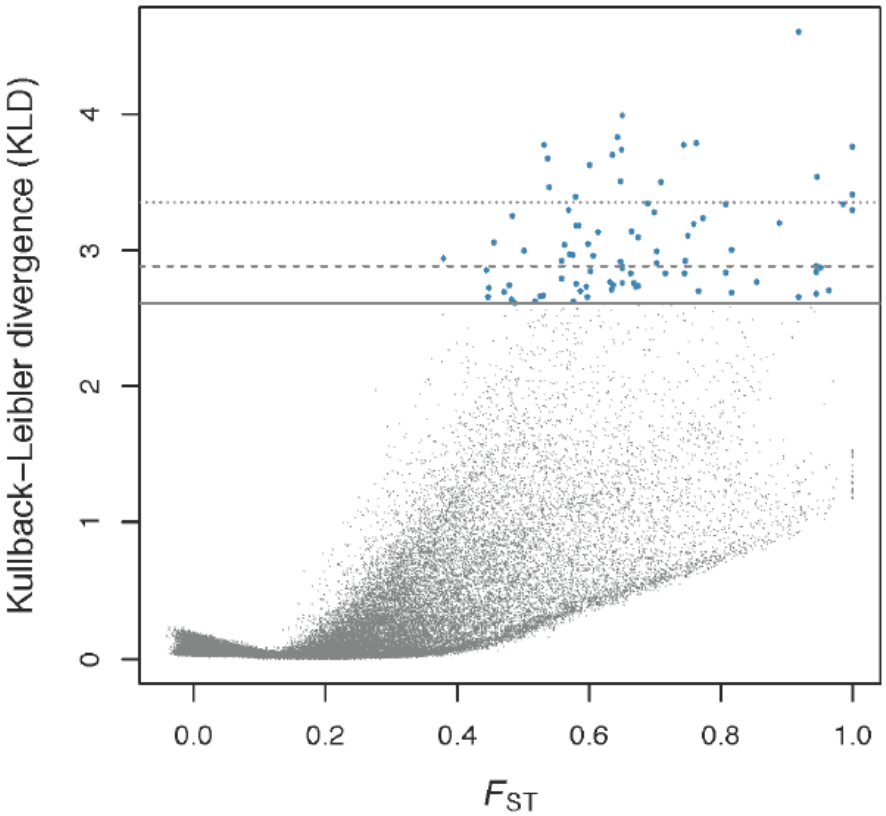


Zeimes et al., 2012



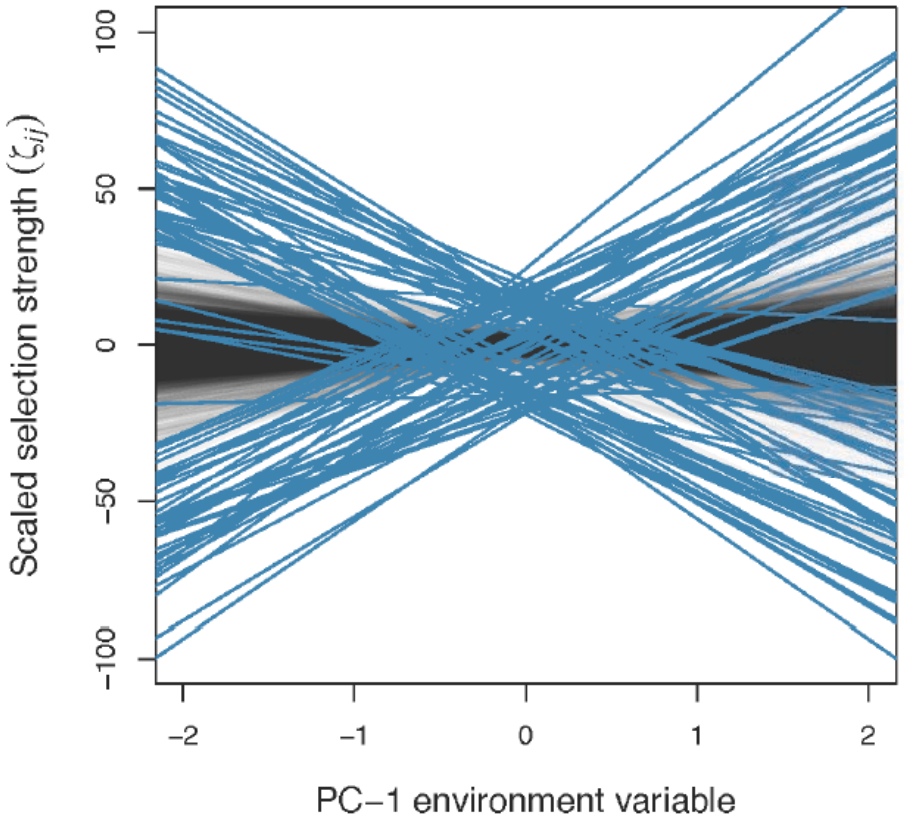
RESULTS

1.a- Genomic Differentiation, SelEstim



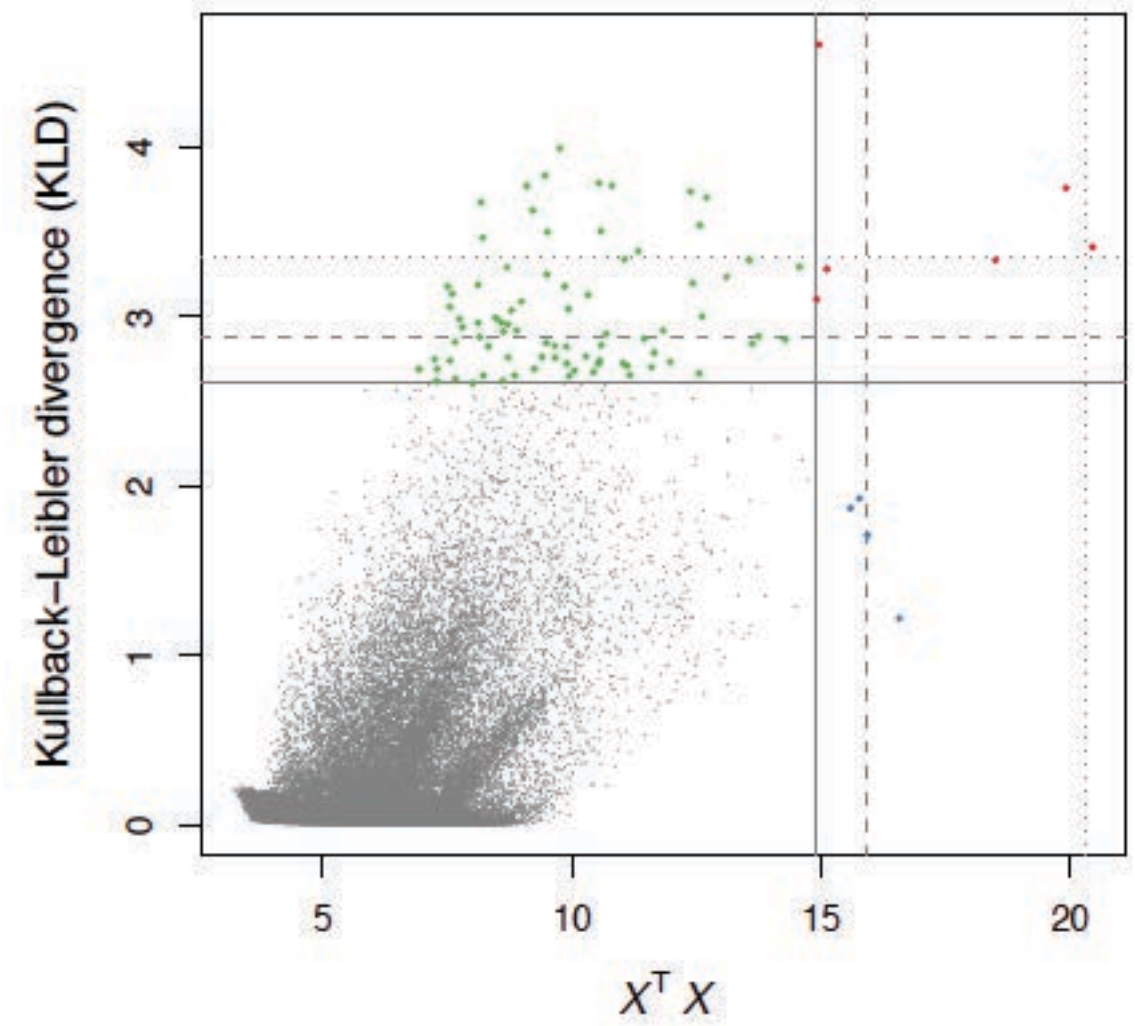
Threshold 99.9%

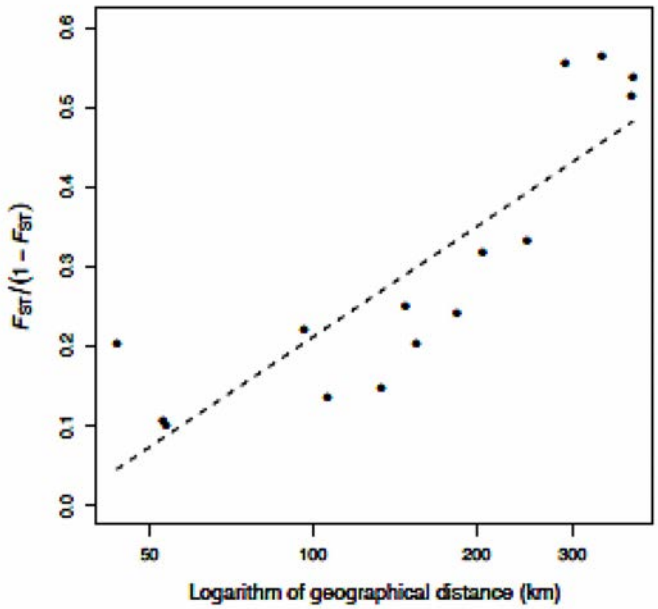
86 outliers detected
Including the candidate *Tlr7*



Strong correlation between
local selection coefficients and
environmental variables associated
with PUUV

1.b- Genomic Differentiation, BayPass



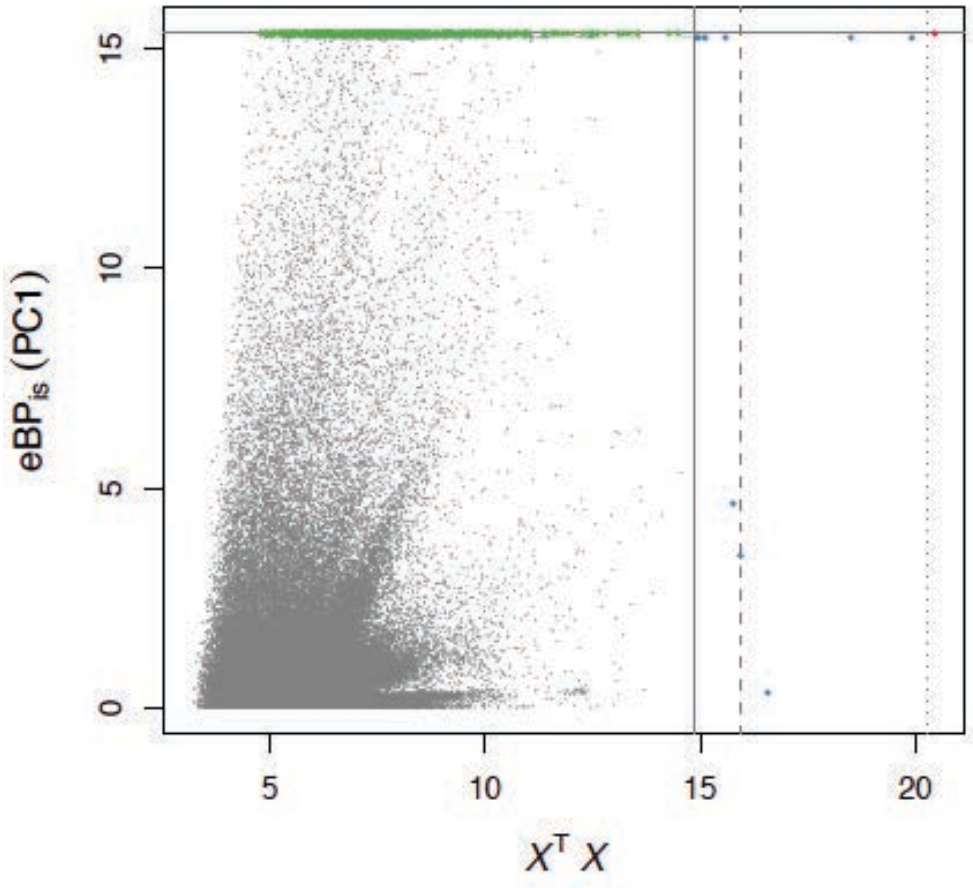


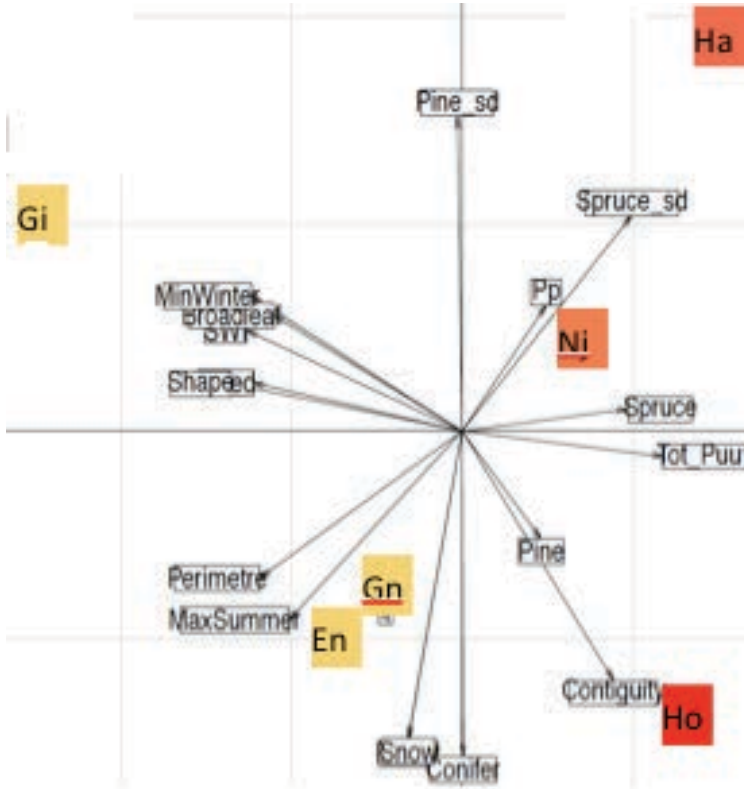
Strong isolation
by distance pattern

Threshold 99.9%

10 outliers detected,
6 are common with SelEstim

2. Environmental covariates, BayPass

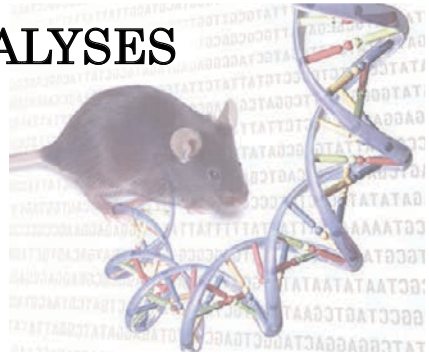




Threshold 99.9%

395 outliers detected with PC1, 26 are common with SelEstim or BAyPAss

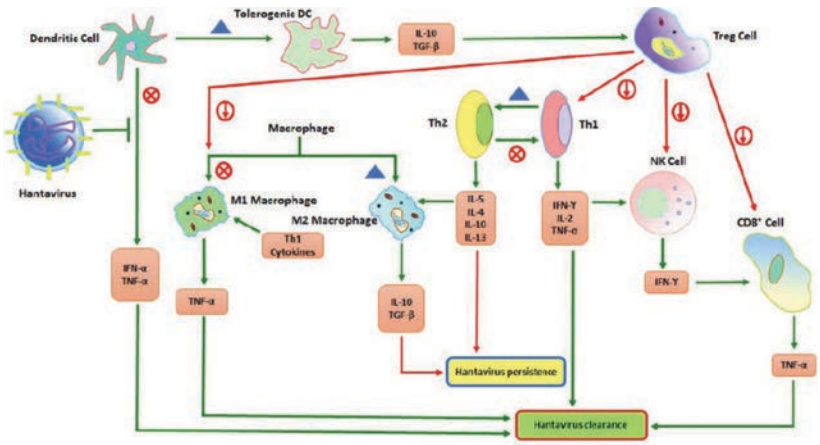
ANNOTATIONS OF OUTLIERS AND GENE ENRICHMENT ANALYSES



Among the 70.000 contigs and using
M. musculus genome

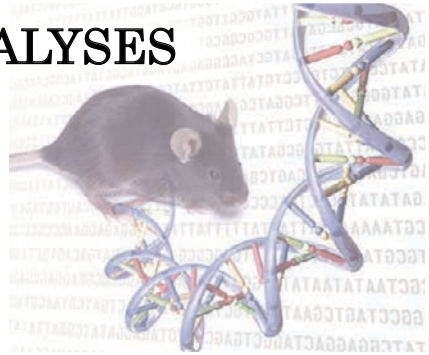
44 % are annotated using BlastN
21 % are annotated using BlastX
14 % matched with coding sequences of proteins

22 chromosomes are covered

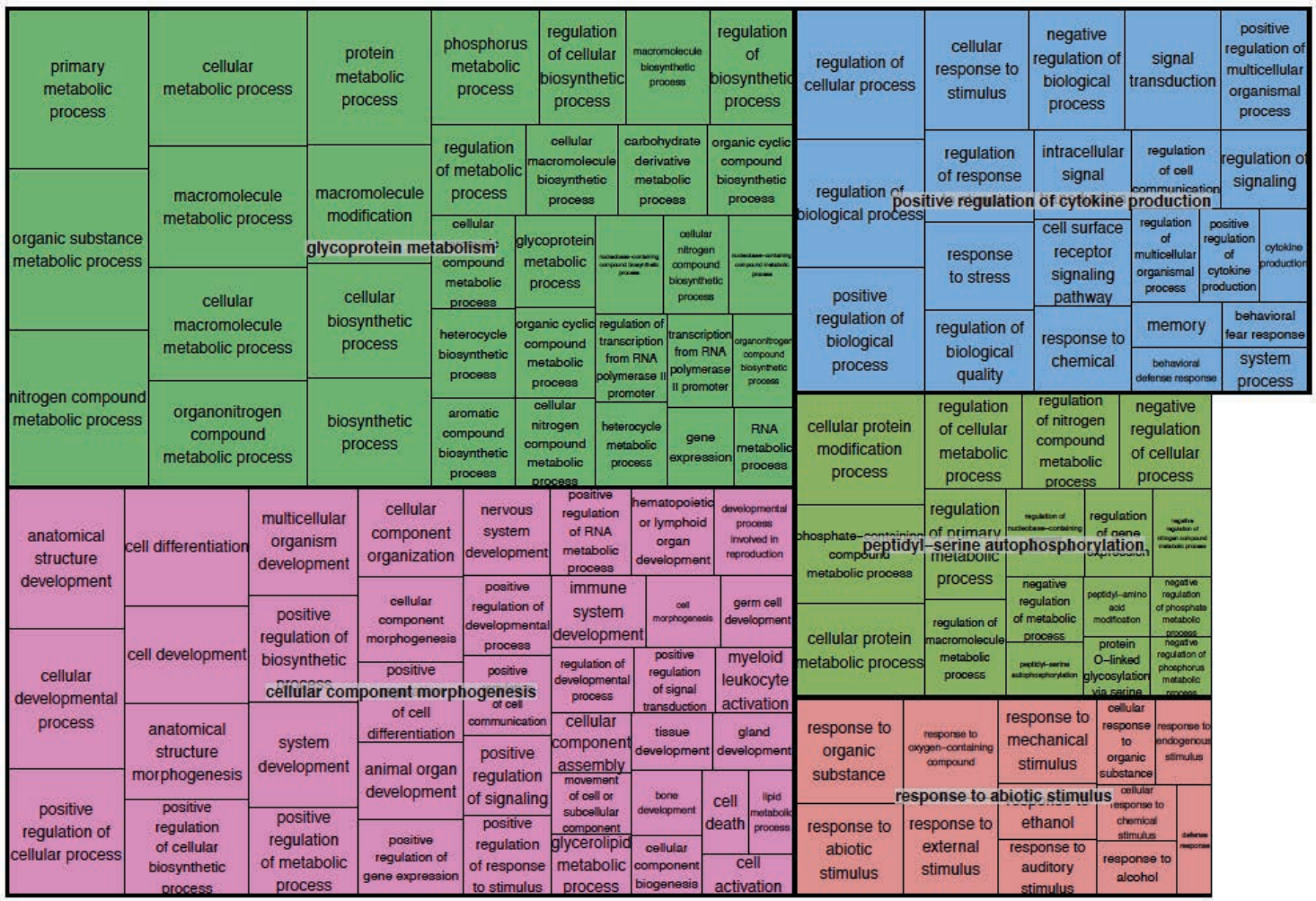


Annotation of outliers
468 RAD – contigs
108 outliers – including *Tlr7* – matched to
104 mouse protein-coding genes
GO annotations for 95 of them
14 genes had GO related with immunity

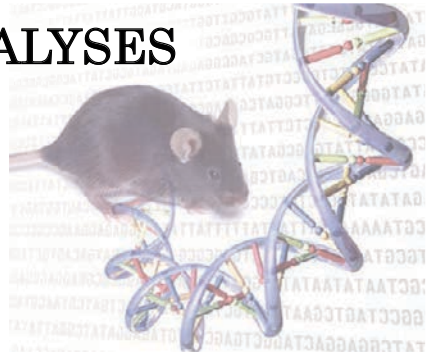
ANNOTATIONS OF OUTLIERS AND GENE ENRICHMENT ANALYSES



The third most represented biological process was the 'positive regulation of cytokine production'



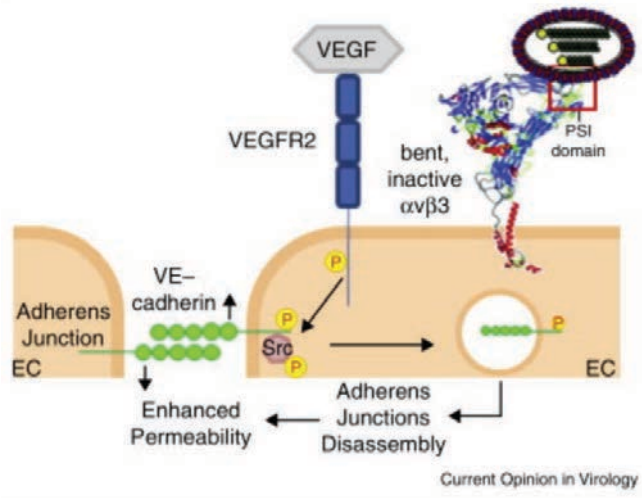
ANNOTATIONS OF OUTLIERS AND GENE ENRICHMENT ANALYSES

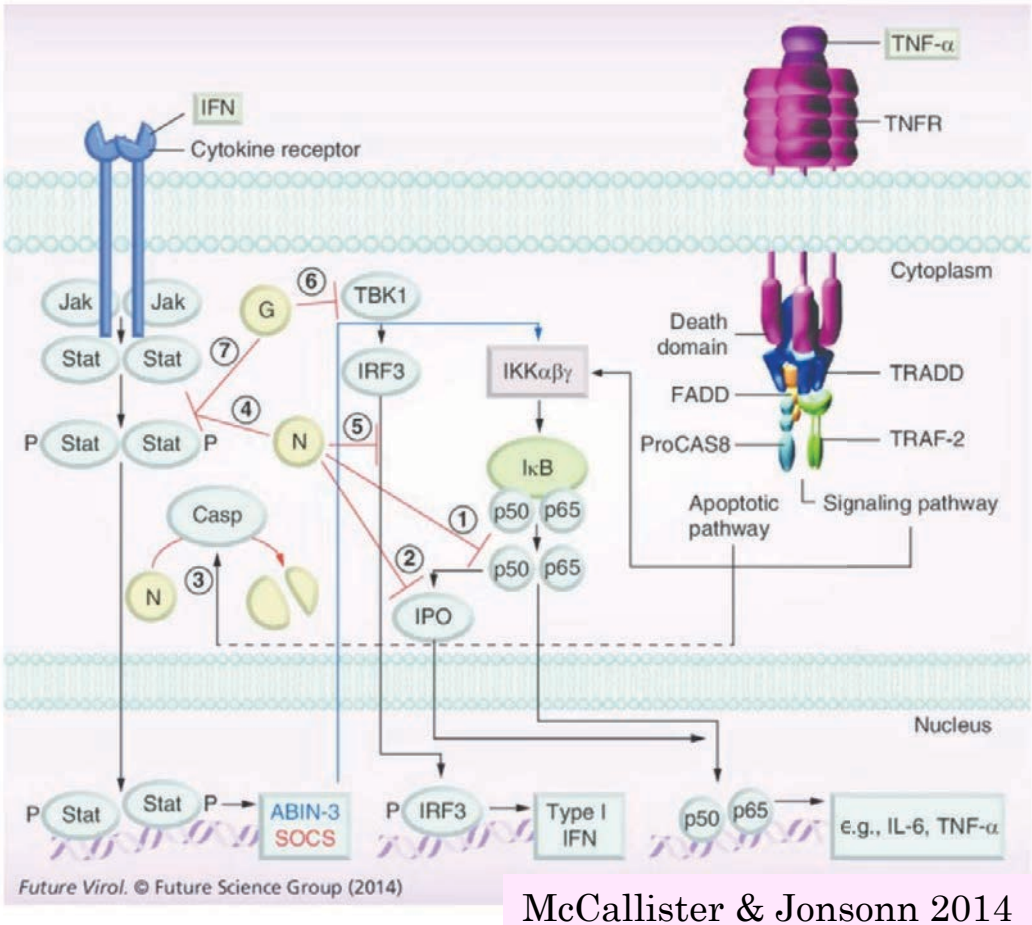


125 pathways were identified,
out of which 13 were significantly enriched

Toll-Like Receptors cascades
JAK STAT pathway regulation
IL-7 signaling

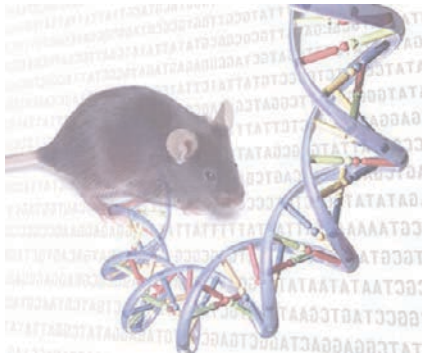
+ VEGF signalling pathway





McCallister & Jonsonn 2014

CONCLUSIONS FROM THE GENOMICS APPROACHES



Limits of the study

- Low number of populations
- High levels of genomic differentiation (contact zone)
- Potential confounding effects (correlation latitude and PUUV distr.)

- Lack of power to annotate outliers

But

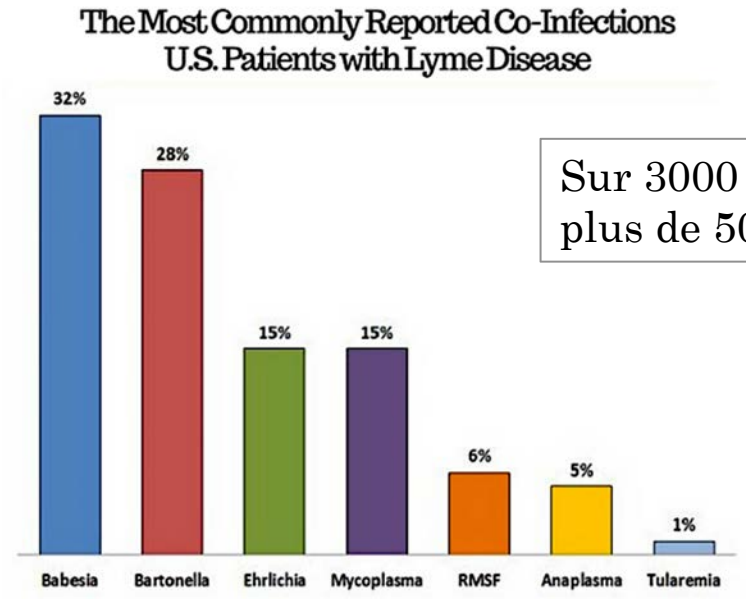
- Outliers showing congruent 'spatial' patterns of selection
- Candidate genes & expected genes with regard to immunity

Perspectives

- Large spatio-temporal surveys of bank vole populations in Sweden
- Analyses of other european samplings (e.g. France, Germany)
- Improvement of bank vole genome / transcriptome annotation
- Collaborations with immunologists, clinicians to interpret outliers

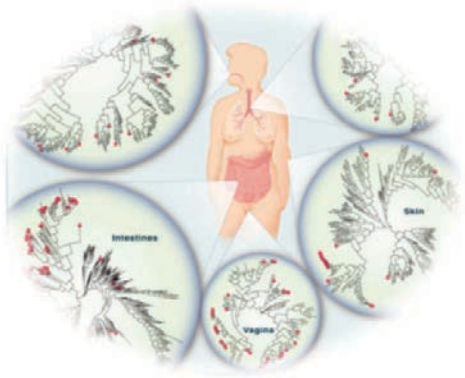
FROM HOST-PARASITE TO HOST PATHOBIOME

Exposition simultanée à de nombreux pathogènes = fréquent
Influence épidémiologique (sensibilité, pathogénicité …)



Sur 3000 patients diagnostiqués pour 'Lyme', plus de 50% ont des coinfections bactériennes

Microbiome = très diversifié, fort impact sur l'immunité

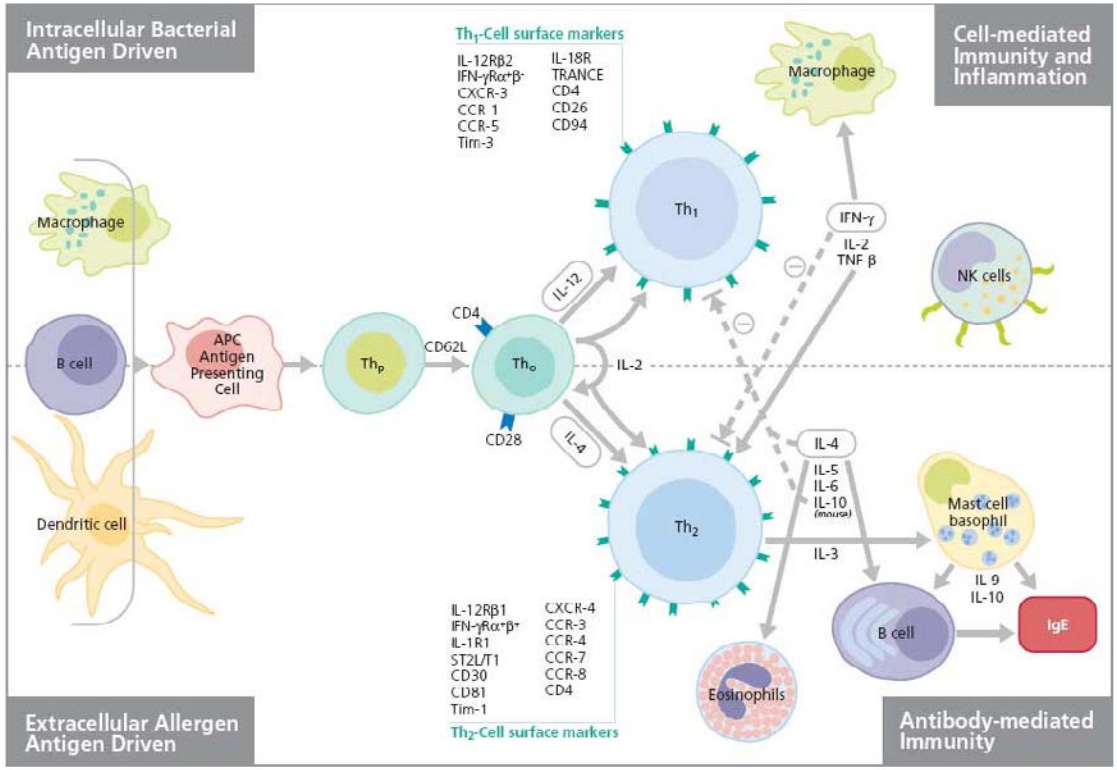


➡ Changement de paradigme
1 hôte / 1 pathogène
Hôte / **Pathobiome =** pathogène et son environnement biotique

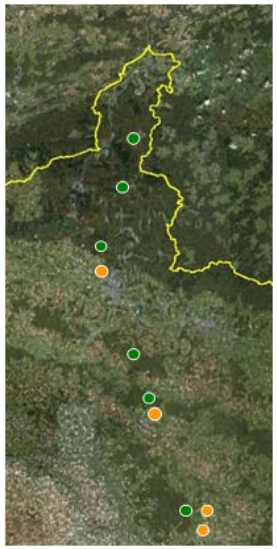
CO-INFECTIONS BETWEEN PUUV AND HELMINTHS

Evolutionary or physiological trade-offs between immune responses

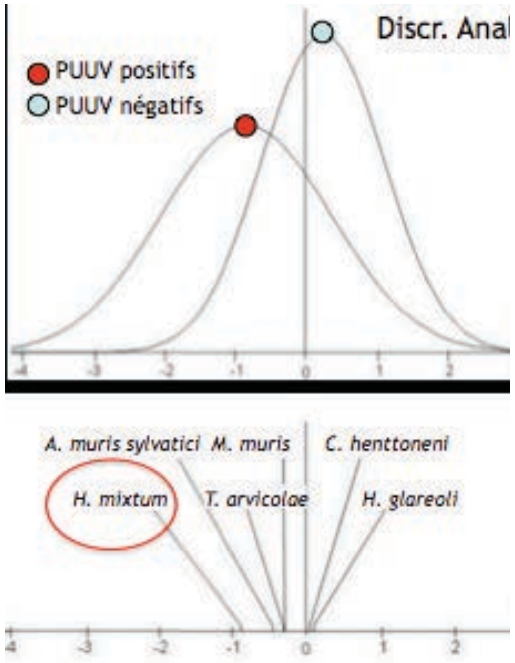
Ex : Antagonistic immune responses to intra & extra cellular pathogens (Th1/Th2)



Hyp : High Th1 (eg inflammation) = low risk of viral infection
but high risk of helminth inf.



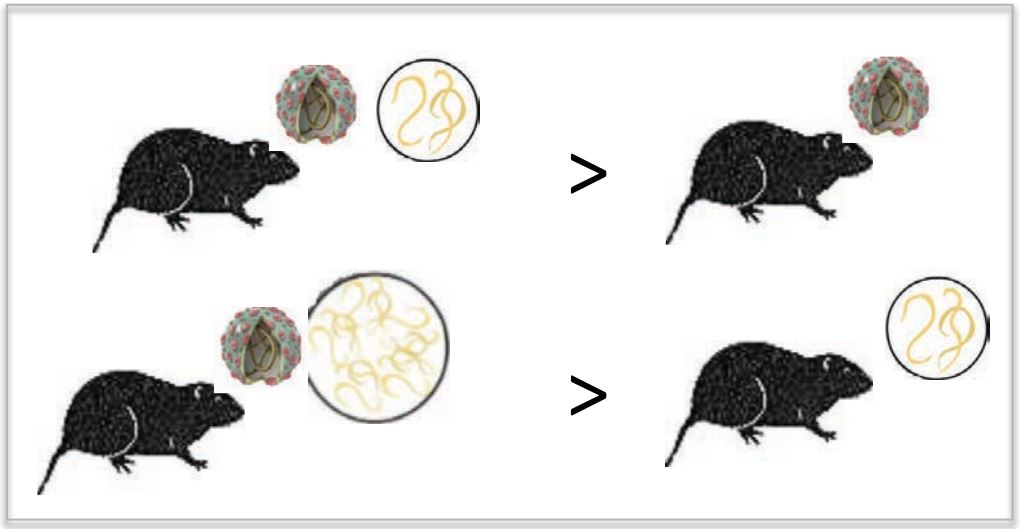
French Ardennes

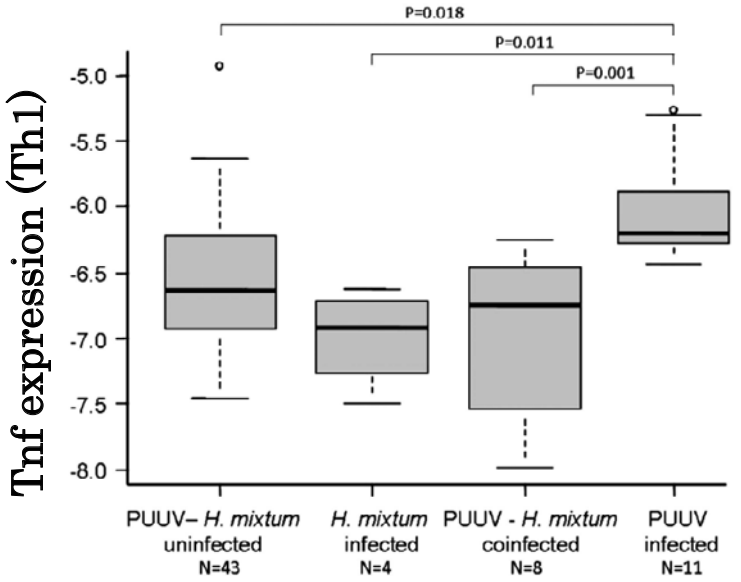


Co-infections *H. mixtum* / PUUV

Co-infection increases *H. mixtum* load

Th1 could mediate these patterns





INTERACTIONS BETWEEN PUUV THE BACTERIOME AND THE MYCOME

Methodological challenges in molecular biology, bioinformatics and biostatistics

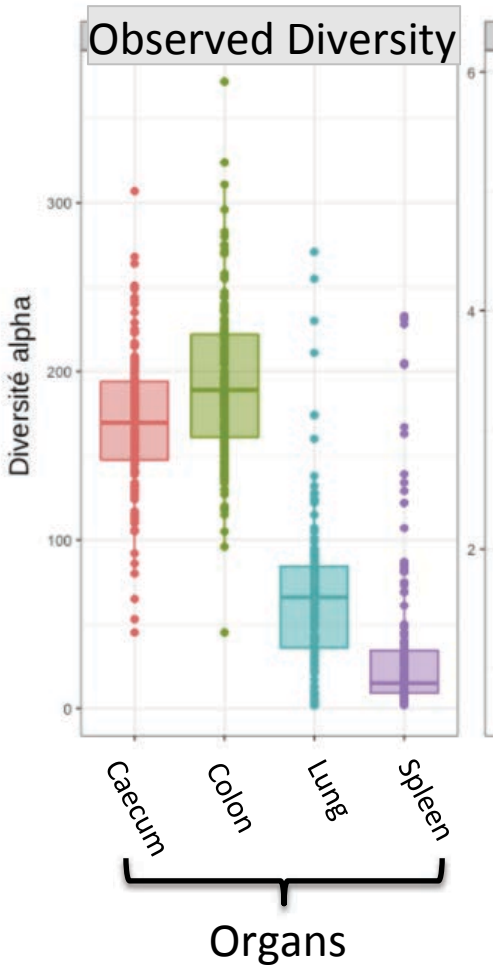
Solved for bacteriome studies

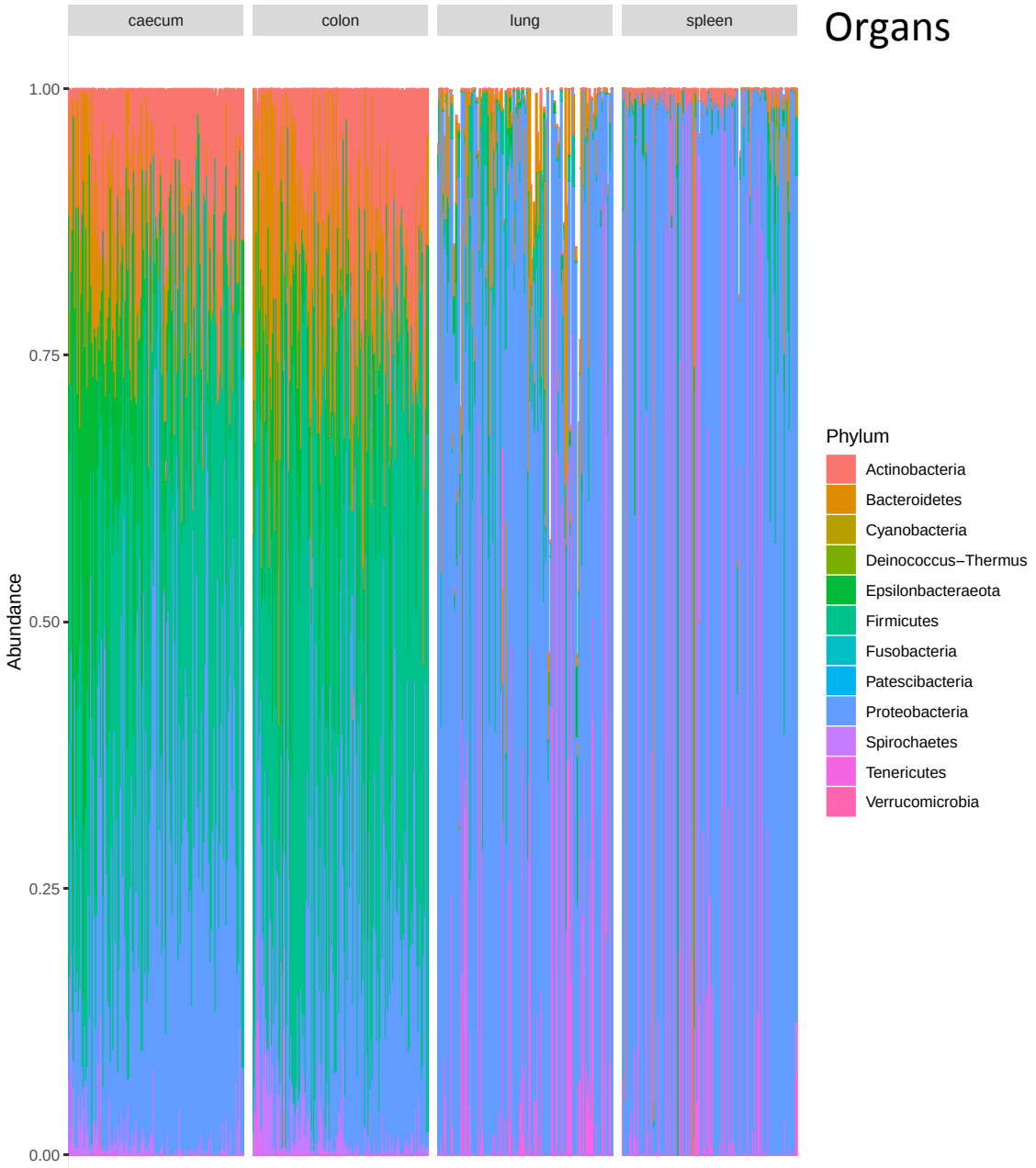
In progress for mycobiome study
(but see FrogsFungi : https://github.com/geraldinepascal/FROGS/releases)

In progress for PUUV intra-host diversity analyses (see Guillaume Castel)

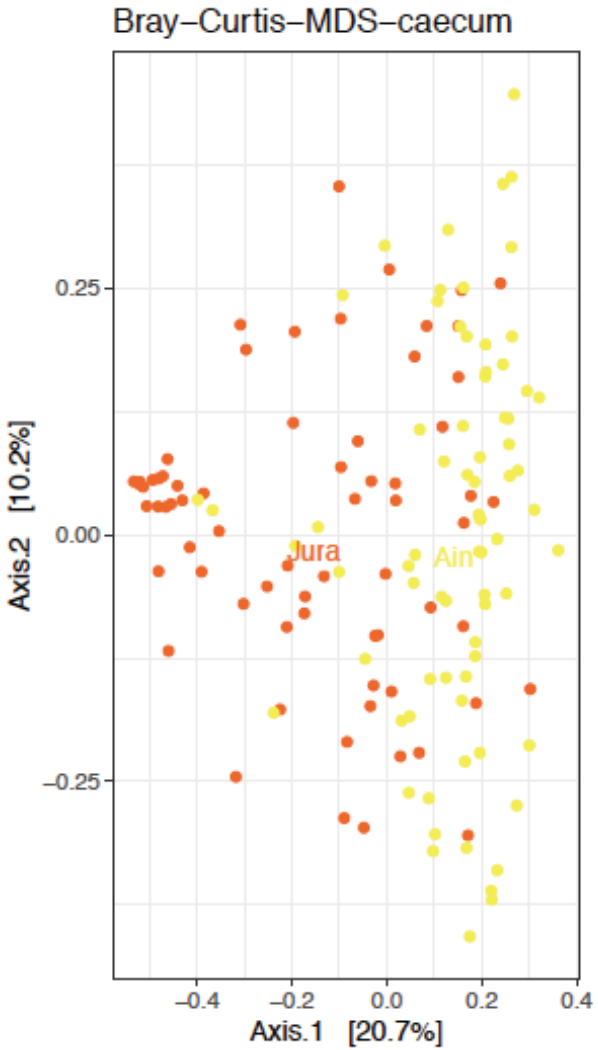
No Hypothesis : 'catch me if you can' strategy !

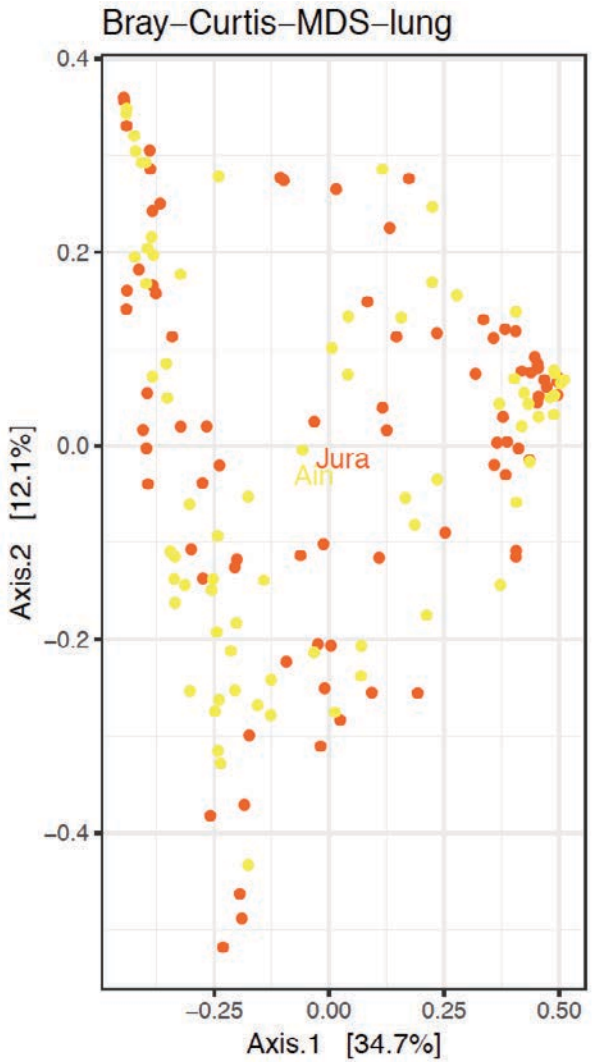
BACTERIOME DIVERSITY AND COMPOSITION





Beta diversity analyses





Differences of bacterial communities between Jura and Ain (Caecum & Colon not Lung or Spleen)

INTERACTIONS BETWEEN BACTERIA AND PUUV

Gut bacterial communities

Analyse how gut bacterial communities change during experimental infections

Analyse interactions between helminths and gut bacteria

Lung and splenic bacteria

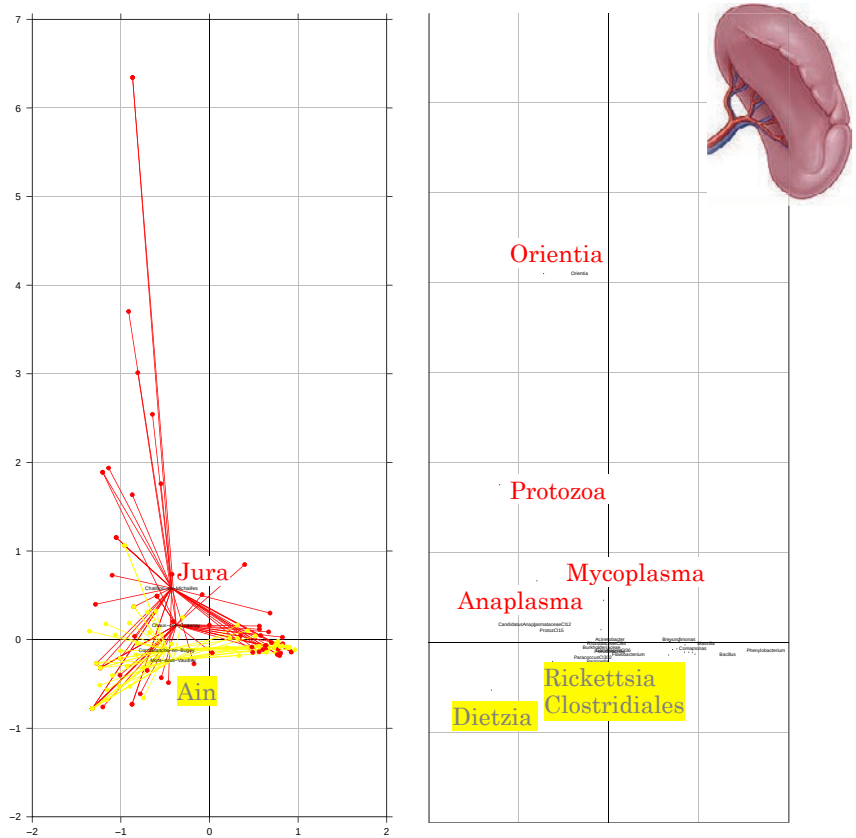
Focus on pathogenic bacteria
&
Detection of associations

Jura / Ain - Spleen

Bartonella, Anaplasma, Rickettsia …

Discriminant analyses on spleen

*** Significant differences between regions



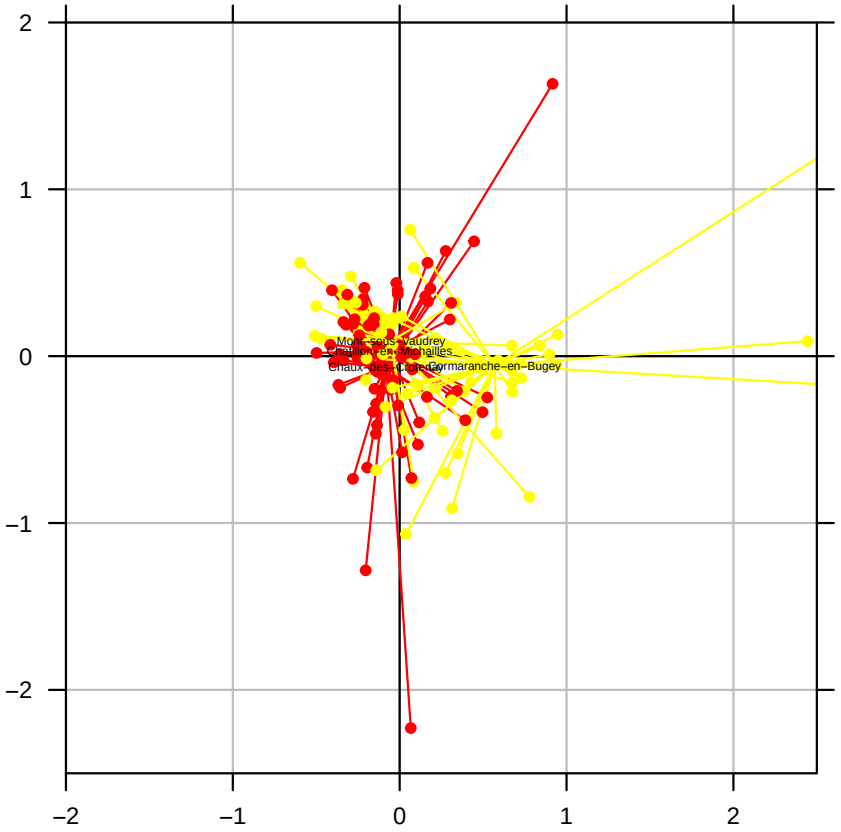
INTERACTIONS BETWEEN BACTERIA AND PUUV

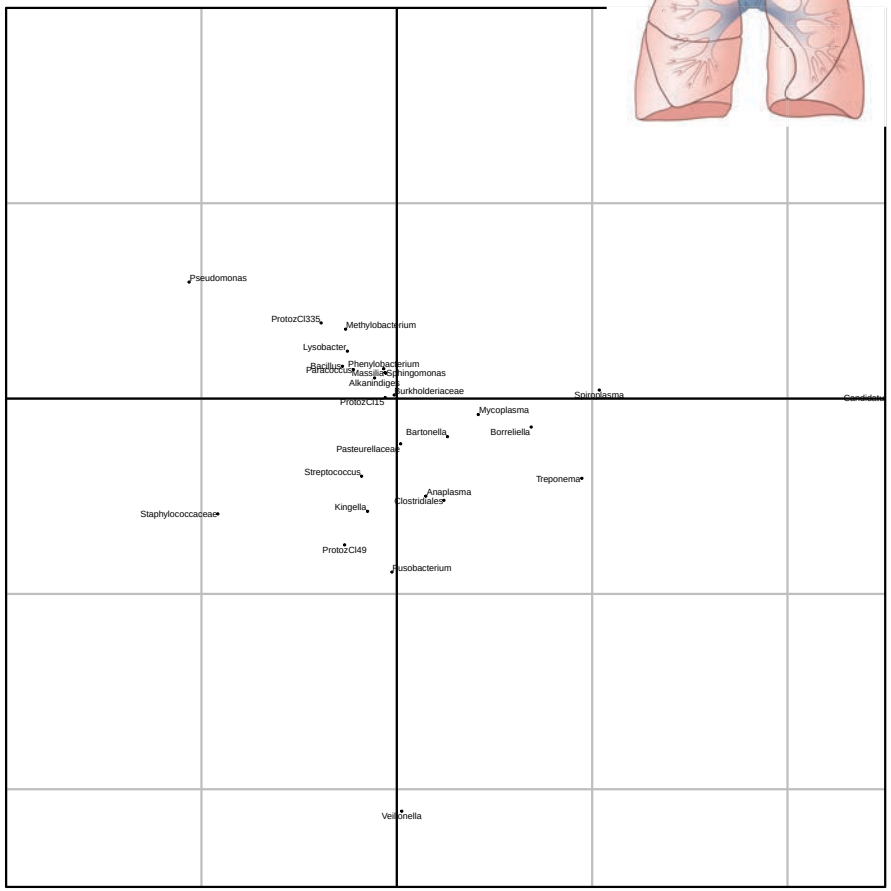
Jura / Ain – Lung

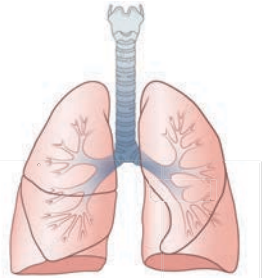
Pasteurellaceae, Pseudomonas, Paracoccus…

Discriminant analyses on lung

No significant differences between regions







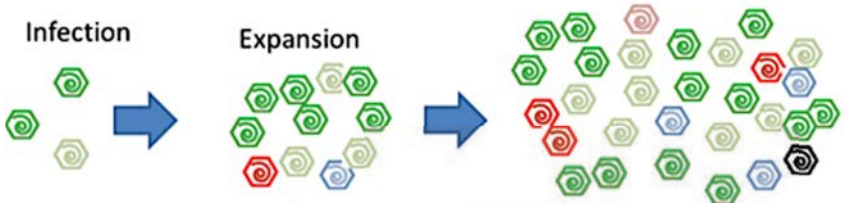
CONCLUSIONS

More information to gather

- Need to evaluate **tolerance**
- Need for a better knowledge of bank vole genome and transcriptome
- …

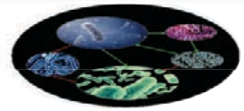
More **integration** is required

Need epidemiologists to **understand** the consequences of the immune/viral variability on PUUV distribution

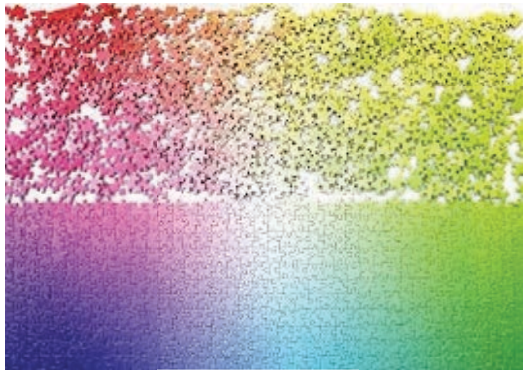


Viral evolution

Pathobiome



Environment, Global changes



Human socio-economics

Host immunity


Anses Lyon
SLU Sweden
Haartman Finland
INRA Gabi, Genphyse
FP6 Eden
FP7 Edenext
INRA EFPA
INRA MEM
ANSES
THANKS FOR YOUR ATTENTION
Photos L. Benoit

Some related publications

Dubois, A., Castel, G., Murri, S., Pulido, C., Pons, J.B., Benoit, L., Loiseau, A., Lakhdar, L., Galan, M., Charbonnel, N., Marianneau, P., 2017a. Experimental infections of wild bank voles (*Myodes glareolus*) from nephropatia epidemica endemic and non-endemic regions revealed slight differences in Puumala virological course and immunological responses. Virus Research 235, 67-72.

Dubois, A., Castel, G., Murri, S., Pulido, C., Pons, J.B., Benoit, L., Loiseau, A., Lakhdar, L., Galan, M., Marianneau, P., Charbonnel, N., 2018. Bank vole immunoheterogeneity may limit nephropatia epidemica emergence in a French non-endemic region. Parasitology 145, 393–407.

Dubois, A., Galan, M., Guivier, E., Henttonen, H., Voutilainen, L., Gauffre, B., Vitalis, R., Cosson, J.F., Charbonnel, N., 2017b. Microevolution of bank voles (*Myodes glareolus*) at neutral and immune-related genes during a multi-annual complete dynamic cycle : consequences for Puumala hantavirus epidemiology. Infection Genetics Evolution 49, 318–329.

Guivier, E., Galan, M., Henttonen, H., Cosson, J.F., Charbonnel, N., 2014. Landscape features - fragmentation and helminth coinfection - shape bank vole immunoheterogeneity, with consequences for Puumala virus epidemiology. Heredity 112, 274-281.

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Ribas Salvador, A., Guivier, E., Chaval, Y., Xuéreb, A., Henttonen, H., Voutilainen, L., Poulle, M.L., Cadet, P., Cosson, J.F., Charbonnel, N., 2011. Concomitant influence of helminth infection and landscape on the distribution of Puumala hantavirus in its reservoir, *Myodes glareolus*. BMC Microbiology 11, 1-13.

Rohfritsch, A., Galan, M., Gautier, M., Gharbi, K., Olsson, G.E., Gschloessl, B., Zeimes, C., VanWambeke, S., Vitalis, R., Charbonnel, N., 2018. Preliminary insights into the genetics of bank vole tolerance to Puumala hantavirus in Sweden. Ecology and Evolution 8, 11273–11292.

Rohfritsch, A., Guivier, E., Galan, M., Chaval, Y., Charbonnel, N., 2013. Apport de l'immunogénétique à la compréhension des interactions entre le campagnol roussâtre Myodes glareolus et l'hantavirus Puumala. Bulletin de l'académie vétérinaire de France 166, 165-176.

Vaumourin, E., Vourc'h, G., Telfer, S., Lambin, X., Salih, D.A., Seitzer, U., Morand, S., Charbonnel, N., Vayssier-Taussat, M., Gasqui, P., 2014. To be or not to be associated: Power study of four statistical modeling approaches to identify pathogen associations in cross-sectional studies. Frontiers in Cell Inf Microbiol 4, 62.